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GENERAL AND PHYSICAL CHEMISTRY.

1. Surface Chemistry.

This section is restricted to certain aspects of monolayers at mobile interfaces which have evoked interest in recent years and have not been dealt with in earlier Reports.[1,2]

The reduction of surface tension by the monolayer, termed the "surface pressure," and previously denoted by F, is now denoted by Π in conformity with the revised nomenclature. The change in phase boundary potential, or "surface potential," is denoted by ΔV and is usually related to the molecular density (n, in molecules/cm.^{2}) by the Helmholtz equation $\Delta V = 4\pi n\mu$, where μ is the vertical component of the apparent dipole moment; $A = 10^{16}/n$, is the area (in A.^{2}) occupied by each molecule in the surface. The measurement of surface pressure, surface potential, and mechanical properties such as elasticity and rigidity have been outlined before.[1,2]

The Structure of Monolayers.—Of the principal two-dimensional phases whose existence is well established, *viz.*, gaseous, expanded, and condensed, the structure in the first two is now generally accepted. Gaseous films approach the ideal relationship $\Pi A = kT$ at very low surface pressures (areas generally of the order 1000—10,000 A.^{2}/molecule), and obey an equation of the Amagat type $\Pi(A - A_0) = xkT$ at higher pressures. Langmuir's explanation for the "expanded" type,[1,3] which have no three-dimensional analogy and which obey the equation of state $(\Pi - \Pi_0)(A - A_0) = C$, has been generally accepted. In this equation Π_0 allows for the (negative) spreading pressure of the chains, A_0 is the "co-area" of the polar head-group, and C a constant equal to kT for un-ionised groups.[4] As pointed out below, this explanation has been strengthened by work upon insoluble monolayers at the oil–water interface.

Considerable discussion still centres, however, upon the condensed films. The Π–A curves of condensed films of simple long-chain hydrocarbon derivatives generally consist of two approximately linear portions, the first and more compressible part extrapolating to about 22—30 A.^{2}, depending upon the nature of the head-group, and the second showing a very low compressibility and extrapolating to 20—21 A.^{2} in all cases. N. K. Adam [5] has ascribed these to close-packed heads and close-packed chains respect-

[1] N. K. Adam, *Ann. Reports*, 1936, **33**, 103.
[2] J. H. Schulman, *ibid.*, 1939, **36**, 94.
[3] I. Langmuir, *J. Chem. Physics*, 1933, **1**, 756.
[4] J. Marsden and E. K. Rideal, *J.*, 1938, 1163.
[5] "Physics and Chemistry of Surfaces," 3rd Edition, 1941 (Oxford Univ. Press).

ively, whereas W. D. Harkins and G. E. Boyd [6] class them as liquid and solid, and D. E. Dervichian [7] claims that breaks occur at areas prescribed by the packing in the various three-dimensional crystalline forms, *e.g.*, at 18·5, 19·5, 20·5, 22, and 23·5 A.^{2} in the case of fatty acids. Dervichian's emphasis upon a close correspondence between the two-dimensional condensed film and the three-dimensional crystal has been strongly criticised. For instance, Harkins and Boyd [8] point out that accurate Π–A curves of fatty acids do not show breaks at his predicted areas, and that the liquid condensed phase cannot be crystalline (as postulated by Dervichian), since their viscosity measurements exactly fit the theory of *liquid* monolayers developed by W. J. Moore and H. Eyring.[9] In the Reporter's opinion [10] the available evidence is strongly opposed to any such general thesis,[11] a conclusion supported by subsequent work. Although a powerful interaction between polar group and the aqueous substrate is necessary to obtain spreading, it seems unlikely that the magnitude and direction of the forces involved would exactly compensate in all cases for those in the crystalline state.

In the very incompressible region the recent alternative suggestion that the long-chains are vertically orientated and close-packed [12] is based upon the agreement between the compressibility of the monolayer and of long-chain hydrocarbons in bulk, and between the cross-sectional areas of the monolayer and of paraffins near their melting points (both 19—20 A.^{2}), *i.e.*, in what is believed to be their most nearly comparable states.[12] This formulation is supported by surface-potential measurements upon ethyl stearate, the apparent surface moment of which can only be satisfactorily explained on the basis of vertically orientated chains.[12]

The structure of the more compressible region of condensed films does not seem to be so definitely established, different factors appearing to be operative with different head-groups.[12] For instance, in some cases, *e.g.*, *p*-hexadecylphenyl derivatives, $C_{16}H_{33}\cdot C_6H_4X$, the limiting area is determined by the " size " of the phenyl nucleus, since with X = ^-OH, $^-NH_2$, or $^-OCH_3$ the limiting area remains constant at *ca.* 25 A.^{2}; in others (*e.g.*, *cis-* and *trans*-unsaturated compounds), the hydrocarbon chain packing seems to be the decisive factor, and in certain others hydrogen-bond formation between the head-groups [10, 13] (*e.g.*, long-chain amides, acetamides, and ureas).

The suggested classification of condensed films as " liquid " or " solid " [6] is also controversial. In the more compressible " liquid " region, all films so far studied behave as Newtonian liquids, the surface viscosity η obeying the equation, $\log \eta = \log \eta_0 + k\Pi$, as predicted by Moore and Eyring [9] from Eyring's theory of the liquid state. It seems reasonable therefore

[6] *J. Physical Chem.*, 1941, **45**, 20. [7] *J. Chem. Physics*, 1939, **7**, 931.
[8] *Ibid.*, 1940, **8**, 129. [9] *Ibid.*, 1938, **6**, 391.
[10] A. E. Alexander, *Trans. Faraday Soc.*, 1941, **37**, 426.
[11] See also A. G. Nasini and G. Mattei, *Gazzetta*, 1940, **70**, 697.
[12] A. E. Alexander, *Proc. Roy. Soc.*, 1942, *A*, **179**, 486. [13] *Idem*, *ibid.*, p. 470.

to term this region "liquid condensed," but the use of the term "solid condensed" for *all* films in the other condensed region is less justifiable. Here some films are quite mobile (*e.g.*, methyl ketones and esters), others definitely very rigid (*e.g.*, heavy-metal soaps, ureas, and amides). The long-chain alcohols, acids (un-ionised),[14] and esters,[15] exhibit anomalous surface viscosity, and this is possibly general for all mobile films in this region, but to term these "solid" seems unnecessarily confusing. With the more rigid films a definite elastic modulus can be obtained by applying a torque to a disc on the surface,[16] or by rotating a disc in the liquid and observing the motion of dust particles on the film.[17]

Spreading and Phase Changes of Monolayers.—By means of the Clapeyron equation in the form $\lambda = T(\partial\Pi/\partial T)(A_F - A_S)$ it is possible to determine the latent heat of spreading (λ) from the crystal to the monolayer.[18] (A_F and A_S are areas occupied by film and solid respectively.) Some very accurate Π–A and Π–T measurements have been carried out recently for this purpose by Harkins and his co-workers, with myristic and pentadecylic acids, and latent heats and entropies of spreading determined.[19]

Such measurements lead naturally to a consideration of the thermodynamics of monolayers, such as the energy, entropy, and heats of spreading and expansion, and the order of phase changes.[7, 20]

There has been much discussion recently concerning the fundamental stability of duplex or multi-layers, such as are formed initially by a mixture of an inert oil containing an amphipathic compound as "spreader." This has some practical bearing upon the use of oil films in antimalarial measures, and in preventing the evaporation of water, as discussed below. With simple long-chain acids, alcohols, etc., it seems that the equilibrium system consists of monolayer and lenses, but with polymerised spreaders, or with certain dyes in the aqueous phase, stable (or metastable) thick films could be obtained.[21] It is probably very significant that in all such cases the interfacial films are rigid. W. A. Zisman[22] has recently made a very thorough study of the spreading of drops of an inert oil containing polar compounds, on the lines first initiated by I. Langmuir and K. B. Blodgett,[23] and as expected, maximum spreading is induced by compounds ionised at the interface (*e.g.*, acids on alkaline solutions).

[14] L. Fourt and W. D. Harkins, *J. Physical Chem.*, 1938, **42**, 897; G. E. Boyd and W. D. Harkins, *J. Amer. Chem. Soc.*, 1939, **61**, 1188; L. E. Copeland, W. D. Harkins, and G. E. Boyd, *J. Chem. Physics*, 1942, **10**, 357.

[15] A. A. Trapeznikov, *Acta Physicochim. U.R.S.S.*, 1938, **9**, 273; 1939, **10**, 65; *Doklady Akad. Nauk. U.R.S.S.*, 1941, **30**, 319.

[16] H. Mouquin and E. K. Rideal, *Proc. Roy. Soc.*, 1927, *A*, **114**, 690; A. A. Trapoznikov and P. Rebinder, *Compt. rend.* (*Doklady*) *U.R.S.S.*, 1938, **18**, 185.

[17] A. E. Bresler and S. E. Bresler, *J. Physical Chem. U.S.S.R.*, 1940, **14**, 1604.

[18] A. Cary and E. K. Rideal, *Proc. Roy. Soc.*, 1925, *A*, **109**, 301.

[19] W. D. Harkins and G. C. Nutting, *J. Amer. Chem. Soc.*, 1939, **61**, 1702.

[20] W. D. Harkins, T. F. Young and G. E. Boyd, *J. Chem. Physics*, 1940, **8**, 954; W. D. Harkins and L. E. Copeland, *ibid.*, 1942, **10**, 272.

[21] E. Heymann and A. Yoffe, *Trans. Faraday Soc.*, 1942, **38**, 408; 1943, **39**, 217.

[22] *J. Chem. Physics*, 1941, **9**, 534, 729, 789. [23] *J. Franklin Inst.*, 1934, **218**, 143.

The kinetics of spreading, both from liquid droplets and from crystals, have also been further examined in some detail. In the usual method the edge of the advancing film is indicated by a suitable inert powder and photographed by means of a ciné camera.[24] Another technique, attractive in that it avoids the possibility of contamination by powders, uses a series of air electrodes, connected to an oscillograph with photographic registration, and arranged so that the spreading film passes radially under each in turn.[25] Spreading at interfaces can also be followed by the latter method.

These experiments show that boundary spreading is in general very rapid indeed compared with dissolution from the interface, even with comparatively soluble substances (see also below). The rapidity of spreading of insoluble oils such as oleic acid has indeed been used to investigate the structure of adsorbed films.[24, 26, 27]

Evaporation through Monolayers.—The influence of boundary layers upon the movement of molecules from one phase to another has been little investigated save for the study of evaporation from a water surface, and this has derived much of its impetus from the very practical problem of retarding evaporation from lakes and reservoirs in arid regions, such as South Africa, Australia, and parts of Russia. Although the practical problem does not appear to have been satisfactorily solved, yet some very interesting results have emerged.[21, 28]

Monolayers alone being used, it is generally agreed that only condensed films are appreciably effective, possibly not so surprising in view of the large energy barrier at the interface. With long-chain alcohols, such as octadecyl, as much as 95% reduction can be obtained at high film pressures. Marked reductions can also be obtained by the use of duplex oil films several μ thick, by using suitable polymers as spreaders.[21] These films, as pointed out above, appear to be reasonably stable in the laboratory, but deteriorate when subjected to atmospheric conditions such as rain, wind, and dust.

Reactions in Monolayers.—The study of reactions at the air–water interface by means of the monolayer technique is due principally to Rideal and his co-workers. Reactions can be followed by change of area or of surface potential, usually at constant Π. In almost all cases a very marked dependence upon the molecular orientation at the interface is observed—a true " steric " factor which may modify the reaction velocity by a power of 10 or more.

[24] *E.g.*, F. Ford and D. A. Wilson, *J. Physical Chem.*, 1938, **42**, 1051; E. H. Mercer, *Proc. Physical Soc.*, 1939, **51**, 561; W. von Guttenberg, *Z. Physik*, 1941, **118**, 22.

[25] A. E. Alexander and T. Teorell, unpublished.

[26] J. W. McBain and W. V. Spencer, *J. Amer. Chem. Soc.*, 1940, **62**, 239.

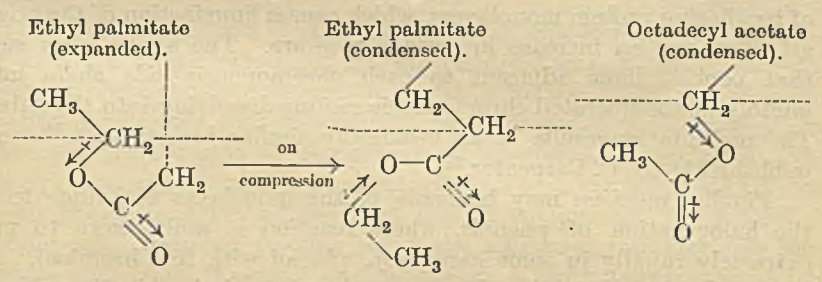
[27] A. E. Alexander, *Trans. Faraday Soc.*, 1942, **38**, 54.

[28] N. I. Glazov, *J. Physical Chem. U.S.S.R.*, 1938, **11**, 484; F. Sebba and H. V. A. Briscoe, *J.*, 1940, 106, 114, 128; F. Sebba and E. K. Rideal, *Trans. Faraday Soc.*, 1941, **37**, 273; A. S. Kheinman, *J. Physical Chem. U.S.S.R.*, 1940, **14**, 118; S. I. Sklyarenko, M. K. Baranaev, and K. I. Mezhueva, *ibid.*, p. 839; I. Langmuir and V. J. Schaefer, *J. Franklin Inst.* 1943, **235**, 119.

The oxidation of unsaturated compounds by dilute permanganate, outlined in an earlier Report,[1] has been further studied with triolein [29] and with *cis*- and *trans*-unsaturated acids.[4] Erucic and brassidic acids show very clearly the greater ease of packing of the hydrocarbon chains in the *trans*-compound (and hence of removal of the double bond from the aqueous phase), since the oxidation of brassidic acid (*trans*) can be almost completely inhibited by increasing the surface pressure, whereas the *cis*-form (erucic) is but little affected.

Closely related to the above, and showing a common sensitivity to molecular orientation, is the oxidation and subsequent polymerisation of monolayers of drying oils, by atmospheric oxygen, ozone, or permanganate.[30] Certain other types of polymerisation, *viz.*, pepsin–formalin, cadaverine–pepsin, and tetra-aminobenzidine–stearaldehyde, have also been followed in monolayers,[31] and it is clear that the method offers considerable scope in this direction.

The hydrolysis of lactones [32] and esters [33] and the lactonisation of hydroxy-acids [34] also provide several points of interest. The alkaline hydrolysis of esters shows particularly well how the velocity of interfacial reactions can be controlled by means of a true steric factor. For example, with an expanded film of ethyl palmitate the short ethyl chain lies on the surface, and the reaction can proceed quite rapidly ($k = 0{\cdot}04$ min.$^{-1}$). On compression to the condensed state the short chain is forced beneath the surface, thus screening the ester group and reducing the velocity constant k to 0·005 min.$^{-1}$. The change can be depicted as below :



$\mu_{obs.}$, milli-Debyes	535	193	502
$\mu_{calc.}$, „ „	525	198	525
A, A.^{2}	>72	20	23
k, min.$^{-1}$	0·04	0·005	0·15

[29] R. Mittelmann and R. C. Palmer, *Trans. Faraday Soc.*, 1942, **38**, 506.

[30] G. Gee and E. K. Rideal, *Proc. Roy. Soc.*, 1935, *A*, **153**, 116, 129; *J.*, 1937, 772; P. H. Faucett, *Drugs, Oils and Paints*, 1939, **54**, 13; A. G. Nasini and P. Ghersa, *Atti X° Cong. intern. Chim.*, 1939, **4**, 236; C. Ockrent and W. H. Banks, *Nature*, 1940, **145**, 861; A. G. Nasini and G. Mattei, *Gazzetta*, 1941, **71**, 302, 422.

[31] S. E. Bresler, D. L. Talmud, and M. F. Yudin, *J. Physical Chem. U.S.S.R.*, 1940, **14**, 801; *Acta Physicochim. U.R.S.S.*, 1941, **14**, 71.

[32] R. J. Fosbinder and E. K. Rideal, *Proc. Roy. Soc.*, 1933, *A*, **143**, 61.

[33] A. E. Alexander and J. H. Schulman, *ibid.*, 1937, *A*, **161**, 115; A. E. Alexander and E. K. Rideal, *ibid.*, 1937, *A*, **163**, 70.

[34] F. Kögl and E. Havinga, *Rec. Trav. chim.*, 1940, **59**, 601.

The validity of these configurations was confirmed by the agreement between the observed surface moments (μ) and those calculated by the Thomson–Eucken vectorial summation method. With a long-chain acetate, on the other hand, it is clear from the above diagram that condensation affords no such protection, and the reaction is still rapid ($k = 0{\cdot}15$ min.$^{-1}$).

The lactonisation of monolayers of hydroxy-fatty acids on acid substrates has shown in one case (β-hydroxyethyloctadecylmalonic acid) a rate some 10^3—10^4 times greater than in solution at the same pH; in another case (γ-hydroxystearic acid) reaction was slower in the film.[34] The latter effect has been ascribed to steric hindrance, whereas the former may be due either to a particularly favourable proximity of hydroxyl and carboxyl groups, or to a higher concentration of hydrogen ions in the surface layer than in the bulk medium. The latter explanation may well be the correct one, since various workers, from other considerations, have suggested just such a difference between surface and bulk pH.[35] It would be interesting to see this finally established by this rather direct method.

The monolayer technique enabled J. S. Mitchell and E. K. Rideal [36] to investigate the effect of ultra-violet radiation upon proteins, using as a starting point monolayers of stearanilide and other simple compounds containing the $-CO{\cdot}NH-$ link. The presence of the aromatic nucleus induced photochemical hydrolysis, leaving a monolayer of stearic acid, at wave-lengths of 2483 A. and less, the apparent quantum efficiency and rate of reaction being very sensitive to the orientation of the aromatic chromophor with respect to the surface. The same authors later [37] described the effect of irradiating protein monolayers, which causes liquefaction of the originally gel films and an increase in surface pressure. The above work suggests that peptide links adjacent to each chromophoric side chain undergo photolysis, the liberated chromophoric residue dissolving into the substrate. The preliminary results of an essentially similar investigation have been published by D. C. Carpenter.[38]

Finally, mention may be made of the monolayer technique to study the halogenation of phenols, where reaction is well known to proceed extremely rapidly in some cases (*e.g.*, phenol with free bromine). Monolayers of *p*-hexadecylphenol were employed, and the kinetics of reaction with hypohalous acid, free halogen, and trihalide ions determined.[39] Reactions with a half-life of as little as 40 seconds could be followed satisfactorily. The halogenation of long-chain unsaturated compounds, particularly by iodine chloride, which is closely related to the above, has been followed in similar manner by A. G. Nasini and G. Mattei.[40]

Insoluble Monolayers at the Oil–Water Interface.—It is only comparatively recently that any marked success has been achieved in the study of insoluble

[35] J. F. Danielli, *Proc. Roy. Soc.*, 1937, *B*, **122**, 155; G. S. Hartley and J. W. Roe, *Trans. Faraday Soc.*, 1940, **36**, 101.
[36] *Proc. Roy. Soc.*, 1937, *A*, **159**, 206.
[37] *Ibid.*, 1938, *A*, **167**, 342.
[38] *Science*, 1939, **89**, 251.
[39] A. E. Alexander, *J.*, 1938, 729.
[40] *Gazzetta*, 1940, **70**, 635.

monolayers at oil–water interfaces. This has been due to the difficulties encountered and not to the lack of interest, since such interfacial films are clearly of more direct relevance to the biologist and emulsion chemist than are those at air–water interfaces.

Some preliminary Π–A measurements were obtained by F. A. Askew and J. F. Danielli,[41] using a slightly modified Langmuir–Adam surface balance, and bromobenzene as the oil phase. Various compounds were measured (egg-albumin, α-aminopalmitic acid, a long-chain amide, and a methyl cellulose), but very great difficulties with leaks and contamination were found.

The various methods which appeared promising, *viz.*, the modified surface balance, detachment methods such as the maximum pull on a ring or frame, and the Wilhelmy plate, were explored by A. E. Alexander and T. Teorell,[42] using benzene as the oil phase, but only the ring method was found suitable. The interface is formed in a large Pyrex dish, and the interfacial tension (γ_0) determined. By means of a micrometer syringe, a small but accurately-known volume of a solution of the compound under examination is then expelled into the interface, and the new interfacial tension (γ) determined as before. Hence the spreading pressure (Π) due to the film can be found, since $\Pi = \gamma_0 - \gamma$. The molecular density at the interface is then increased, and the Π–A curve constructed, not by reducing the area as with air–water monolayers, but by further injections of spreading solution. This technique avoids errors due to leaks and varying contact angles, and gives an accuracy equal to that of the usual film balance for air–water monolayers.

By this means accurate Π–A curves for six compounds at the benzene–water interface were determined, *viz.*, for gliadin, serum albumin, lecithin, lysolecithin, kephalin,[43] and sodium cetyl sulphate. The results show interesting similarities and differences when compared with those given by the same compounds at the air–water interface. For example, the Π–A curves are of the vapour expanded type in all cases, as might be expected from the presence of the oil medium, but on compression the molecules reach the same ultimate packing as at the air–water interface (see also Heymann and Yoffe),[21] indicating that the oil is eventually squeezed out completely from the interface. Films of the above phospholipoids at the air–water interface are of the liquid expanded type, obeying Langmuir's equation of state $(\Pi - \Pi_0)(A - A_0) = C$, and this was also found to hold for their interfacial films. The change from an air–water to a benzene–water interface had no effect on A_0 or C, but eliminated Π_0 almost completely (*e.g.*, from 12·8 to 0·05 dyne/cm. with lysolecithin). As pointed out above, these results provide direct support for Langmuir's theory of expanded films at air–water interfaces.

The mechanical properties of interfacial films (*e.g.*, elasticity and viscosity),

[41] *Proc. Roy. Soc.*, 1936, *A*, **155**, 695; *Trans. Faraday Soc.*, 1940, **36**, 785.
[42] *Ibid.*, 1939, **35**, 727.
[43] A. E. Alexander, T. Teorell, and C. G. Åborg, *ibid.*, p. 1200.

can be determined by an oscillating needle,[44] or by the rotating disc,[45] the methods developed for air–water interfaces being followed closely, but the determination of the change in phase-boundary potential (ΔV) is not yet entirely satisfactory. The difficulty here arises from the high resistance of even a thin film of benzene or other suitable non-polar oil. Some preliminary results for lecithin,[44] using a very thin film of benzene, a large polonium air electrode, and a Lindemann or valve electrometer, indicate that for a given molecular density the dipole orientation and change in phase boundary potential are the same at both air–water and oil–water interfaces.

When a suitable technique for interfacial potentials has been finally evolved, a systematic examination of insoluble interfacial monolayers will become possible. The oil–water shows certain advantages over the air–water interface: spreading is facilitated (*e.g.*, proteins spread readily and completely), and ionised substances, such as soaps and detergents, are stabilised sufficiently to make measurements feasible. A clear understanding of interfacial monolayers should help considerably towards the elucidation of certain problems of adsorbed films.

Some Applications of Monolayer Techniques to Other Fields.—No survey of recent advances in surface chemistry would be complete without outlining some of the ways in which the study of monolayers has assisted problems in other branches of science. In addition to those mentioned above and certain physical aspects recently reviewed,[46] there are numerous applications to organic and physical chemistry, to classical colloidal systems such as emulsions, and finally to biology, a field in which the obvious applicability of surface studies is only just beginning to make itself manifest.

(a) *Structure of complex organic molecules.* The use of monolayer measurements as an aid towards elucidating the structure of complex organic molecules is well shown by several recent publications. The possibilities inherent in such a method had been indicated by the early work of Adam and his co-workers on sterols,[5] and in general terms it can be said that the technique can indicate not only the *general shape* of the molecule but also the relative positions of the polar groups. (One polar group at least is necessary to ensure suitable spreading.) For example, a molecule composed of a complex ring system with two polar groups in close proximity would probably give a condensed film, thus allowing a direct comparison between the monolayer area and that calculated from models of likely structures. If, however, these polar groups are widely separated, then the molecule tends to lie flat on the surface, giving a gaseous or expanded film.

Recent work on these lines has dealt with cerin, friedelin, and related compounds,[47] lupane derivatives,[48] the constitution of quillaic and oleanolic

[44] A. E. Alexander, *Trans. Faraday Soc.*, 1941, **37**, 117.
[45] P. F. Pokhil, *J. Physical Chem. U.S.S.R.*, 1939, **13**, 301.
[46] A. E. Alexander, *Reports Prog. Physics*, 1943, **9**, 158.
[47] N. L. Drake and J. K. Wolfe, *J. Amer. Chem. Soc.*, 1940, **62**, 3018.
[48] P. Bilham, E. R. H. Jones, and R. J. Meakins, *J.*, 1941, 761.

acids,[49] and the position of the carboxyl group in certain triterpene acids.[50] S. Ställberg and E. Stenhagen, in a series of papers,[51] have dealt with monolayers of compounds with branched hydrocarbon chains, with the object of elucidating the structure of phthioic acid [52] and other complex biological compounds.

In this connection it is important to emphasise the value of the multilayer technique for inducing crystallisation of complex organic molecules,[53] often intractable to ordinary methods, so that additional information from *X*-ray analysis might become available.

(b) *Emulsions.* Two fundamental papers [54] upon the formation, stability, and structure of oil-in-water and water-in-oil emulsions were based upon monolayer studies at the air–water interface as detailed in an earlier Report.[2] With some two-component monolayers, such as cholesterol and sodium cetyl sulphate, a marked molecular association, or " complex " formation, is found, as evidenced by the reduction of the surface or interfacial tension, and owing to this extremely low oil–water tension very stable emulsions are formed on shaking a mixture of cholesterol in oil and sodium cetyl sulphate in water. A charged interfacial monolayer, as given by the above system for example, was found to promote oil-in-water emulsions, whereas on discharge, as by addition of calcium ion to an ordinary soap, rigid films are produced which tend to link the oil droplets together, enclosing the aqueous phase and so forming the invert water-in-oil type.

(c) *The surface ageing of solutions.* One phenomenon which has aroused considerable discussion during the past decade is the anomalously slow rate of transfer of molecules from bulk solution to a freshly formed interface, generally termed " surface ageing." [55] Soaps and synthetic detergents often require several days for equilibration if their concentration is below that for micelle formation; if above it, equilibration is very rapid. Unionised amphipathic compounds such as alcohols and acids also show the same slow accumulation, the anomaly increasing with the chain length.

A recent examination from a new angle shows that the time factor is reduced by the presence of simple capillary-active substances (*e.g.*, ethyl acetate), and eliminated at an oil–water interface, even when the oil phase is only of molecular dimensions (*e.g.*, oleic acid or ethyl laurate monolayers).[56] These results appear to rule out earlier theories, which postulated electrostatic potential barriers at the surface [57] or the formation of surface pellicles

[49] P. Bilham and G. A. R. Kon, *J.*, 1941, 552.

[50] P. Bilham, G. A. R. Kon, and W. C. J. Ross, *J.*, 1942, 35.

[51] *Svensk Kem. Tidskr.*, 1940, **52**, 223; 1941, **53**, 335; *J. Biol. Chem.*, 1941, **139**, 345; 1942, **143**, 171; 1943, **148**, 685.

[52] N. Polgar and Sir R. Robinson, *J.*, 1943, 615.

[53] For recent reviews, see refs. (2) and (46).

[54] J. H. Schulman and E. G. Cockbain, *Trans. Faraday Soc.*, 1940, **36**, 651, 661; T. P. Hoar and J. H. Schulman, *Nature*, 1943, **152**, 102.

[55] For early references, see ref. (5).

[56] A. E. Alexander, *Trans. Faraday Soc.*, 1941, **37**, 15.

[57] K. S. G. Doss, *Curr. Sci.*, 1935, **4**, 405; 1937, **5**, 645; *Kolloid Z.*, 1939, **86**, 205; cf. G. S. Hartley and J. W. Roe, *Trans. Faraday Soc.*, 1940, **36**, 101.

more than unimolecular in thickness,[58] and it seems that the slow, rate-determining step is the penetration, into the surface layer, of the hydrophobic portion of the molecule.

A slow accumulation is also observed with polar compounds in non-aqueous media, both to the surface [59] and to an aqueous interface.[60] In these organic media it would appear that the apparent retardation arises primarily from the low concentration of solute present in the monomeric form, since media favouring dissociation, such as nitrobenzene, show much less anomaly than benzene or *cyclo*hexane, where association is almost complete.[61]

Closely related to the above is the remarkable stability of interfacial monolayers of relatively soluble substances (*e.g.*, sodium cetyl sulphate), and the slow rate of re-solution of an adsorbed monolayer on compression.[62] It would thus appear that with the amphipathic type of molecule both entrance into, and escape from, an interface are markedly hindered processes.

(d) *Structure of proteins and of protein monolayers.* The question of the structure of protein monolayers, which, despite a large amount of investigation is by no means settled, and may assist in unravelling the structure of globular and fibrillar proteins, has been approached recently from two new angles.

Since the protein molecule appears to function normally only in the presence of water, it seemed natural to examine the properties of its characteristic $-CO\cdot NH-$ group, with its tendency for hydrogen bonding,[63] in the presence of an aqueous medium. Accordingly, monolayers of long-chain compounds containing this group, such as amides, acetamides, and ureas, were compared with those given by acetates, esters, and methyl ketones which clearly cannot associate by intermolecular hydrogen bonding of the type $>C{=}O\cdots H{-}N<$. The differences were most striking, and it seems that cross hydrogen bonding plays a very important part in condensed monolayers of compounds containing the $-CO\cdot NH-$ group, tending to bring about condensation and solidification.[13] The molecular packing and the mechanical properties were also very sensitive to the pH of the substrate, and the effects of other reagents such as urea and thiocyanates, also known to have a marked influence on native proteins, are being studied.

The other method of approach [64] has been the study of monolayers of linear polymers such as polyacrylates, polymethacrylates, nylons, etc., the last being of considerable interest in view of their close similarity to the polypeptide chain. All these compounds have known structures and this has led to a clearer appreciation of the various factors which contribute towards the overall behaviour of protein monolayers.

[58] J. W. McBain and L. H. Perry, *Ind. Eng. Chem.*, 1939, **31**, 35.
[59] *Idem*, *J. Amer. Chem. Soc.*, 1940, **62**, 989.
[60] A. F. H. Ward and N. Tordai, *Nature*, 1944, **154**, 146.
[61] A. E. Alexander and E. K. Rideal, *ibid.*, 1945, **155**, 18.
[62] A. E. Alexander, *Trans. Faraday Soc.*, 1942, **38**, 54.
[63] W. T. Astbury, *ibid.*, 1940, **36**, 871.
[64] D. Crisp and E. K. Rideal, *Proc. Roy. Soc.*, *A* (in the press).

(e) *Some biological applications.* J. H. Schulman and E. K. Rideal [65] have used the technique of penetration to form mixed monolayers,[2] in particular cholesterol with saponin or various synthetic detergents, in an investigation of hæmolysis and agglutination. They have also [66] examined the adsorption on to protein monolayers of homologous series of biologically active compounds (*e.g.*, œstrogenic stilbene derivatives), and related these to their known biological effects.

The mechanism of fat absorption in the intestine has been studied by A. C. Frazer, H. C. Stewart, and J. H. Schulman,[67] using emulsion systems based upon the monolayer and emulsion work mentioned above.

Finally, mention may be made of investigations into the anthelmintic and anti-bacterial action of soap–phenol mixtures, in which the biological activity is in general accelerated by low concentrations of soap, but inhibited by high concentrations. Surface studies [68] show that the acceleration arises from complex formation between soap and phenol, enhancing the surface activity of the mixture. The acceleration is a maximum at the critical concentration for micelles, since at higher soap concentrations competition between soap micelles and biological interface brings about progressive inhibition of biological activity.

It is hoped that this necessarily brief outline has indicated some of the types of problem which can be assisted by surface studies. A. E. A.

2. Some Physicochemical Aspects of Bacterial Growth.

Introduction.

Bacteria are unicellular, without differentiated organs. In suitable media they grow and multiply by binary division. They perform a wide variety of chemical feats in virtue of enzymes which are sometimes isolable from the cell but are more often integral parts of the structure. The cell contents are not homogeneous, but the internal differences are of a fine-grained order.[1,2,3] The material of the cell is largely a network of macromolecular substances, the most important of which are proteins, permeated by an aqueous solution in which substances of lower molecular weight can diffuse about. Such a system presents certain possibilities, suggested by analogy with non-living systems, and one may well wonder to what extent these possibilities in fact help to explain the mechanisms which the cell actually uses.

We will begin by mentioning certain analogies, and then, in the light of them, consider some of the phenomena of bacterial growth.

In the first place, it is probable that the various enzyme functions of a cell

[65] *Proc. Roy. Soc.*, 1937, *B*, **122**, 29.
[66] *Nature*, 1939, **144**, 100. [67] *Ibid.*, 1942, **149**, 167.
[68] A. E. Alexander and A. R. Trim, *ibid.*, 1944, **154**, 177.
[1] G. Knaysi and S. Mudd, *J. Bact.*, 1943, **45**, 349.
[2] G. Piekarski, *Z. Bakt.*, 1939, **144**, 140.
[3] C. F. Robinow, *Proc. Roy. Soc.*, 1942, *B*, **130**, 299.

depend upon specific protein textures, and that these textures are built up during growth by heterogeneous polycondensation reactions not wholly unlike those with which chemical kinetics has already made us familiar. In the cell, moreover, there is a complex scheme of linked processes, in which starting materials, often of the simplest kind, are built up stepwise to give cell material. There must be a whole series of reactions so linked that the products of one enzyme process diffuse from one region of the cell to another, there to participate in further enzyme processes.

Cells reproduce themselves: all individual enzymes must therefore increase in amount during growth—and, to a first approximation, in a constant ratio. This suggests the scheme for the fundamental growth reaction:

enzyme + substrate =
expanded enzyme + products available for further cell reactions

In other words the enzymes expand as they do their work. There are analogies for this kind of reaction in other parts of chemistry. The decomposition of arsine is catalysed by arsenic according to the scheme: solid arsenic + arsine = more solid arsenic + hydrogen. The essential basis for the catalysis here is the tendency of the regular lattice of the solid arsenic to expand by the accretion of like units.[4] This principle is rather important. Proteins and other macromolecular structures constitute ordered arrays, and when they expand by the addition of fresh fragments—after the manner of crystal growth—there will be a release of free energy. This can compensate decreases elsewhere, so that other products of great chemical activity, possibly free radicals, could be released, capable of taking part in further polycondensation reactions in other parts of the cell.

The concentration in which intermediate products reach the next enzyme of a sequence depends upon the spatial distribution of matter in the cell. This applies even if the intermediates are not labile, since, if they are of low molecular weight, the process of loss from the cell by diffusion will be in competition with that of utilisation by other enzymes.

Thus we have the picture of a sequence of consecutive reactions, with relative rates determined by spatial, as well as temporal factors, occurring in different regions between which transit of intermediate products is governed by the establishment of definite concentration gradients. These reactions lead to the expansion of various ordered arrays of macromolecular compounds. One problem confronting us is this: which, if any, of the characteristics of the bacterial cell are understandable in terms of the chemical kinetics of spatio-temporally linked reaction sequences, and, indeed, in terms of relative reaction rates of different enzyme processes? A few of the outstanding phenomena of bacterial growth will be discussed from this point of view.

The first group of facts relate to the so-called *growth cycle*.[5] In a sequence of reactions of the kind envisaged, if all raw materials are supplied

[4] C. N. Hinshelwood, *J.*, 1939, 1203. [5] Cf. Topley and Wilson, "Bacteriology."

from a constant environment, a steady state will be established in which all the regions of the cell material expand at rates such that their relative proportions remain unchanged. For some reason, unspecified at present, the cell divides when it exceeds a certain size. Each cell can go on growing at the same rate as its parent. Hence the total number increases with time in geometrical progression according to the law $n = n_0 e^{kt}$, where n_0 is the original number and n the number at time t, k being a constant. This law is, in fact, rather closely followed (with understandable deviations) over a quite wide range of growth known as the *logarithmic growth phase*. Ultimately the supply of raw material becomes exhausted, or substances are formed which inhibit some stage or stages of the reaction sequence, and growth ceases. The cells may go on living but they enter what is called the *stationary phase*. During this, some of the intermediate products of the sequence are lost by decomposition or diffusion: more profound changes,

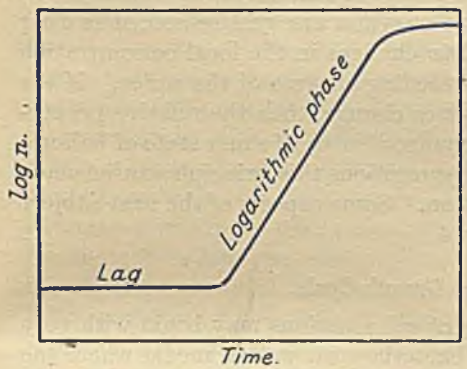


FIG. 1.

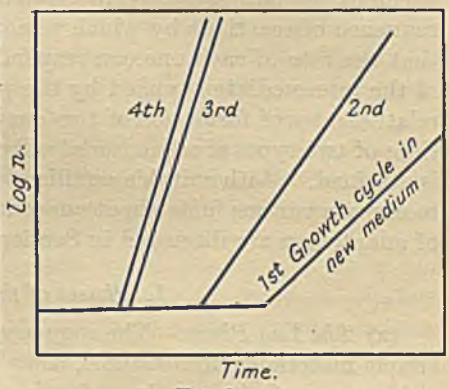


FIG. 2.

possibly involving structural alterations in the proteins, may also occur. The result is that even if the cells are transferred to fresh media there may be a delay, known as the *lag phase*, before the steady state necessary for growth and division is re-established. The phases of the growth cycle are shown in Fig. 1. (If the stationary phase is prolonged the cells die : the death rate will not be considered at present.) In Section 1 we shall deal briefly with various factors governing the length of the lag phase, the rate of growth in the logarithmic phase, and the onset of the stationary phase.

Having considered the initiation and maintenance of growth, we should logically proceed to discuss cell division. This can be done at present only in an imperfect manner, and it is not clear what is the appropriate physicochemical model. However, various interesting and suggestive facts have come to light which will be dealt with in Section 2.

The various enzymes concerned in the sequence of growth processes are the seat of reactions possessing many characters in common with surface reactions, and which must be susceptible to inhibition by substances having affinity for them. Enzymes will also be inhibited by agents which damage

them structurally or deprive them of their substrates. These factors form the basis of various forms of drug action, some of which are considered in Section 3.

One of the most striking characteristics of bacteria is their power of adaptation. When cells which have been grown for some time in a given medium are transferred to a new one in which the necessary carbon or nitrogen is supplied in the form of compounds not present in the original medium, growth may be slow during the first few growth cycles, but gradually increase in rate in the manner illustrated (in a slightly idealised way) in Fig. 2. Similarly, in the presence of certain drugs there may initially be a very marked deceleration of growth, but after a few growth cycles the cells become immune and grow quite unhindered by the drug. This remarkable behaviour, apparently so typical of a living organism, can, hypothetically, be explained in terms of an automatic adjustment of the enzyme balance in the cell : it becomes, in fact, a study in relative reaction rates. If we consider the sequence of reactions by which various enzymes are synthesised, it is clear that the rate of each one can respond to changes in the local concentration of the intermediate supplied by the preceding enzyme of the series. If the relative rates of formation of two enzymes change, then the relative proportions of two types of cell material will change also, until a new state of balance is attained. With suitable auxiliary assumptions this principle can be made to explain various facts about adaptation. Some aspects of the vast subject of adaptation are discussed in Section 4.

1. *Phases of the Growth Cycle.*

(a) *The Lag Phase.*—The sequence of cell reactions may begin with very simple materials; for example, some bacteria grow well in media where the only source of nitrogen is ammonia and the only source of carbon a simple compound like glycerol. The compounds built up are of much greater complexity, and some organisms will not start to grow unless they are supplied with molecules of various specific kinds ready made.[6] Among necessary growth substances for various bacteria are glutamine,[7] tryptophan,[8] uracil,[9] nicotinic acid, thiamin and various amino-acids.[6] Similar compounds are therefore likely intermediates in the chain of processes occurring in cells which can start with simpler materials. Indeed, certain bacteria which normally demand tryptophan, an essential constituent of the protein, can adapt themselves to build it up, first from indole and then from ammonia.[8,10,11] Hence the order, ammonia, indole, tryptophan is indicated, and it depends simply upon the state of the cell enzymes whether the earlier

[6] B. C. J. G. Knight, "Bacterial Nutrition," 1936.

[7] H. McIlwain, P. Fildes, G. P. Gladstone, and B. C. J. G. Knight, *Biochem. J.*, 1939, 33, 223.

[8] P. Fildes, G. P. Gladstone, and B. C. J. G. Knight, *Brit. J. Exp. Path.*, 1933, 14, 189.

[9] G. M. Richardson, *Biochem. J.*, 1936, 30, 2184.

[10] P. Fildes and B. C. J. G. Knight, *Brit. J. Exp. Path.*, 1933, 14, 343.

[11] P. Fildes, *ibid.*, 1940, 21, 67.

stages operate or whether they have to be by-passed. Very simple substances indeed may be necessary for growth. One of the most interesting is carbon dioxide. When a stream of air freed from this is passed through cultures in some media, growth is delayed indefinitely.[12, 13, 14] Many cells also require inorganic ions; for instance, when glucose is used as carbon source in presence of inorganic phosphate, the length of the lag phase may depend markedly upon small concentrations of magnesium ions.[15] Some substances used in growth are probably broken down in one department of the cell and passed on to another department for further processing. When *Staphylococcus aureus* adapts itself to grow without ready-made alanine, it can simultaneously dispense with various other amino-acids which it normally demands.[16] This may mean that the mechanisms which are mobilised deal, not specifically with the individual amino-acids, but with active fragments common to them all.

During the early stages of the lag phase there is no apparent increase in cell substance; later the cell volume increases and this is usually heralded by an increased production of metabolites such as carbon dioxide.[17, 18, 19] One of the obvious conclusions is that during the lag there must be established the necessary concentrations and concentration gradients of the less complex intermediates. This is confirmed by the fact that the lag in simple artificial media is longer than in media like meat extract which contain a varied stock of ready-synthesised compounds. The simpler intermediates are diffusible substances which not only pass from one part of the cell to another, but may be easily lost into the surrounding medium. This comes to light in the quantitative study of the lag phase. We may take the example of *Bact. lactis ærogenes*, which grows easily with glucose as carbon source and ammonium sulphate or amino-acids as nitrogen source. The following experiment is made : cells from a growing culture are transferred to a fresh supply of the same medium and the length of the lag is determined (cf. Fig. 1). The lag proves to be a function of the age of the parent cells, *i.e.*, the time between start of growth of the parent and the transfer to the new medium. When amino-acids are the source of nitrogen, the result is as shown in Fig. 3, but with ammonium sulphate as source the relation illustrated in Fig. 4 is found.[20] The explanation of the minimum is as follows. In the ammonium sulphate medium a diffusible intermediate escapes into the solution. Ordinarily cells transferred to a new medium carry with them a certain volume of the original medium. In Fig. 4 the initial fall of the lag to a minimum is due to the increasing amounts of the intermediate transferred

[12] G. P. Gladstone, P. Fildes, and G. M. Richardson, *Brit. J. Exp. Path.*, 1935, **16**, 335.
[13] S. Dagley and C. N. Hinshelwood, *J.*, 1938, 1936.
[14] W. Kempner and C. Schlayer, *J. Bact.*, 1942, **43**, 387.
[15] R. M. Lodge and C. N. Hinshelwood, *J.*, 1939, 1692.
[16] G. P. Gladstone, *Brit. J. Exp. Path.*, 1937, **18**, 322.
[17] G. Mooney and C.-E. A. Winslow, *J. Bact.*, 1935, **30**, 427.
[18] E. Huntingdon and C.-E. A. Winslow, *ibid.*, 1937, **33**, 123.
[19] C.-E. A. Winslow and I. J. Walker, *Bact. Rev.*, 1939, **3**, 147
[20] R. M. Lodge and C. N. Hinshelwood, *J.*, 1943, 213.

with the cells. Actual filtered medium from the old culture lowers the lag of a new culture. The lag of freshly transferred cells which have been washed free of the old medium is much increased. Moreover, with the washed cells the lag depends in a marked degree on the actual number transferred, since they all form the diffusible intermediate and pour it into the medium to form a common store, and the more there are to contribute their quota the sooner is the critical concentration built up. A fairly satisfactory quantitative theory of the lag under such conditions can be constructed with the aid of the assumptions (i) that the lag ends when the concentration, c, of some active substance in each cell reaches a critical value, c'; (ii) that c is made up in three ways, being expressed by $c = \alpha v + \beta n_0 t + \gamma t$, where v is the volume of the old medium transferred, n_0 the number of cells transferred, and α, β, and γ are constants. The first term represents the active substance transferred with the old medium accompanying the cells, the second that built up by them in time t in the new medium, and the third that built up in a given

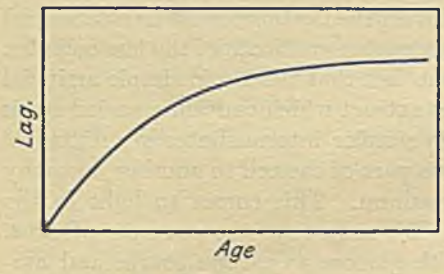


Fig. 3.

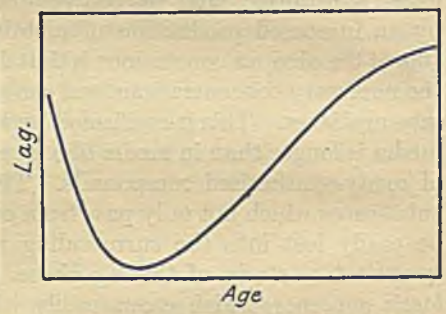


Fig. 4.

cell without help from the medium or from the other cells. The resulting expression for the lag gives a reasonable account of its variation with separate changes in v and n_0 (which can be varied independently with washed cells). The variation of lag with n_0 is specially striking: it is not found when amino-acids are used instead of ammonium sulphate, since under these conditions loss of the intermediate into the medium plays a less important part.

Various qualitative references to the influence of the inoculum size on the lag occur in the earlier literature.[5] Diffusible substances which leave the cell play a part in the functioning of various specific enzymes such as deaminases, and dehydrogenases under conditions where these are not directly involved in growth.[21, 22, 23] To what extent the increases in lag on continued ageing of the cells (Figs. 3 and 4) are due to simple decay or loss of active intermediates, and to what extent due to actual structural changes in the protein, is imperfectly known.

The lag is a function of the concentrations of medium constituents, but does not vary rapidly with that of the ordinary food materials. It is

[21] J. Yudkin, *Biochem. J.*, 1937, **31**, 865.
[22] E. F. Gale and M. Stephenson, *Biochem. J.*, 1938, **32**, 392.
[23] D. D. Woods and A. R. Trim, *ibid.*, 1942, **36**, 501

specifically influenced by various drugs, and this influence is easily modifiable by adaptive changes in the cells in a way which will be considered later.

(b) *The Logarithmic Growth Phase.*—At the end of the lag phase the steady state is established, and the rate of increase of all the cell substance at any moment becomes proportional to the amount present, *i.e.*, $dm/dt = km$. In so far as division occurs when a standard size is reached (which is only roughly true), this means that the number of cells increases in geometrical proportion with time, the number doubling in equal increments of a period usually called the mean generation time. The constancy of the mean generation time is an approximation : small deviations arise from several causes—consumption of the food materials, accumulation of inhibitors, non-survival of some of the newly formed cells, and variation in the mean size of cells formed at different stages.[21] Quantitatively, however, these

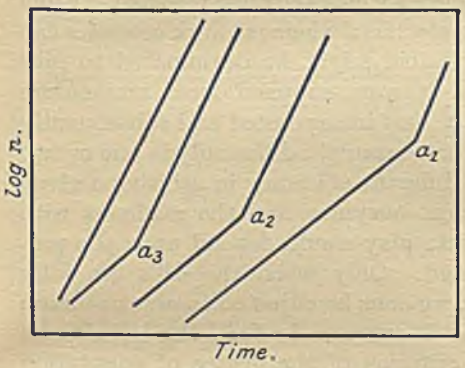


Fig. 5.

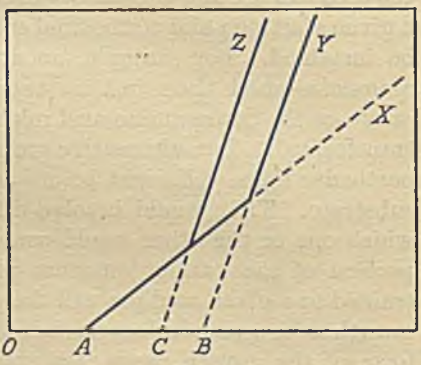


Fig. 6.

effects are usually quite small until the logarithmic phase approaches its end. One or more of them then rapidly becomes serious and growth becomes slower and stops,[25] often with what appears as considerable abruptness.

The actual growth rate of a given organism varies widely, at least over a tenfold range, according to the nature of the medium. The mean generation time, at a given temperature, depends upon the relative amounts of the various cell enzymes and upon the nature and concentration of the substrates provided. The first factor seems to govern the extraordinary way in which cells modify themselves to utilise a given new substrate, a process called training or adaptation. The course of the training process is instructive and is illustrated in Fig. 5. *Bact. lactis œrogenes* accustomed to use glucose as a carbon source is grown in a medium where the glucose is replaced by glycerol. At first the growth rate is low, but increases suddenly at a certain stage of the logarithmic phase as at the point a_1.[26] When the experiment is repeated with cells already grown once in the new medium, this point occurs successively earlier, at a_2, a_3 . . . and finally passes below the range

[24] A. T. Henrici, *Proc. Soc. Exp. Biol. Med.*, 1922, **20**, 179; 1923, **21**, 215, 343, 345.
[25] C. N. Hinshelwood, *Biol. Rev.*, 1944, **19**, 135.
[26] R. M. Lodge and C. N. Hinshelwood, *Trans. Faraday Soc.*, 1944, **40**, 571.

of observation. The simplest interpretation of this is given in Fig. 6. The cells may be assumed to utilise the new medium by two mechanisms, one with a slower growth rate but a shorter lag, the other with a higher growth rate but a longer lag (as shown by the lines AX and BY, respectively). The latter represents a mechanism which utilises glycerol more efficiently but is not originally in a state of mobilisation. The training consists in the progressive reduction of the lag phase of this competing mechanism, as shown by the movement of the second part of the growth curve from the position BY to that CZ. The existence of what appear to be alternative and competing mechanisms comes to light in other connexions, *e.g.*, in training to utilise new nitrogen sources, or to resist the action of sulphonamide drugs.[27] Any mode of growth involves a complex sequence of reactions, and the variety of possible links in any such chain is shown by the number of substrates which the cells can use and the variety of reactions which they can provoke. From a given starting point to the final cell materials a whole network of routes can be imagined. For example, an amino-acid might be deaminated to give ammonia—used then just as though it were supplied from ammonium salts—or the entire amino-acid might be first incorporated and subsequently transformed. Two alternative growth modes might differ only in one or two particular links, yet might be of very different efficiency in utilising a given substrate. They would involve different enzymes, and the readiness with which one or the other would come into play would depend upon the proportion of the various enzymes present. Only when the cells are fully trained to a given medium will the proportions be adjusted to give optimum growth and a simple logarithmic growth curve. (In fact, the logarithmic form of the growth curve is a good criterion of the degree of adaptation and of the extent to which the various cell processes are in balance.)

Although the rates of particular reactions and the combination of reactions used in the total growth sequence are modifiable by training, there are well-defined limits to the adjustments possible. When adaptation has gone to its limit the slowest step determines the overall rate of growth. The fact that there is probably a single rate-determining step is relevant to the question of the relation between rate and nature of substrate. The mean generation time of *Bact. lactis ærogenes* varies little for a series of amino-acids as nitrogen sources, so that presumably the step involving their utilisation is not a rate-determining one, though in some other respects the behaviour of different amino-acids may be very divergent.[28, 29] On the other hand, the rate of growth may vary widely according to the nature of the carbon source. For one representative bacterium, at least, the mean generation time has been shown to be almost independent of the medium pH—which is rather remarkable in view of the profound influence which

[27] D. S. Davies and C. N. Hinshelwood, *Trans. Faraday Soc.*, 1943, **39**, 431.

[28] S. A. Koser and L. F. Rettger, *J. Infect. Dis.*, 1919, **24**, 301; M. Sahyun, P. Beard, E. W. Schultz, J. Snow, and E. Cross, *ibid.*, 1936, **58**, 28; J. Gordon and J. W. M'Leod, *J. Path. Bact.*, 1926, **29**, 13.

[29] R. M. Lodge and C. N. Hinshelwood, *J.*, 1943, 208.

pH has on cell activities in general.[30] Experimental data on the relation of growth rate to concentration of medium constituents are not very abundant. On the whole, however, it appears that over wide ranges of concentration the growth rate varies little, and only shows a marked falling off when the concentration of the foodstuff becomes very small indeed.[30, 31, 32] This can be understood. The cell reactions resemble heterogeneous reactions, and their rates are probably related to concentration of substrate by an equation like that of an adsorption isotherm, rate $= kc/(1 + bc)$, where c is the concentration and k and b are constants. As soon as c exceeds a certain value, this expression becomes nearly constant, in a way familiar in the study of surface reactions. Since the scale of cell processes is a minute one, the saturation limit may very well be reached early. (This is one of the reasons why the mean generation time remains nearly constant over the logarithmic phase despite the consumption of food material.)

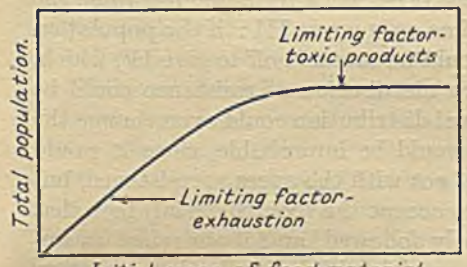


Fig. 7.

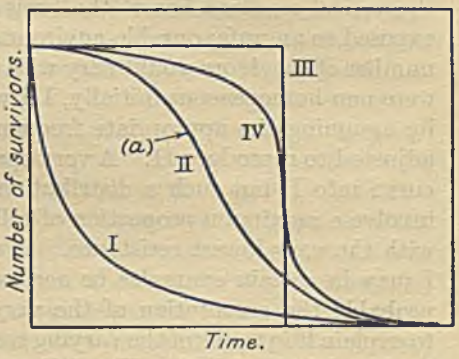


Fig. 8.

(c) *The Stationary Phase* : *Total Cell Population which the Medium will Support.*—One or other of several factors may, according to circumstances, be the limiting one in determining the end of the growth phase and the magnitude of the final population. When exhaustion of foodstuff is the limiting factor, the population is directly proportional to the initial concentration of one of the medium constituents. Sometimes this linear relation holds over a certain range and then breaks down at concentrations where accumulation of toxic products becomes limiting, as shown in Fig. 7.[33]

The effects on the total final population of growth inhibitors added to the medium are various. One might have expected the inhibitors to lengthen the mean generation time without affecting the final state : this, however, is seldom found. Alcohols,[34] for example, not only decrease rate of growth but reduce the final population in nearly the same ratio. Adverse pH reduces the final population without much affecting the rate at which

[30] R. M. Lodge and C. N. Hinshelwood, *J.*, 1939, 1683.
[31] W. J. Penfold and D. Norris, *J. Hyg.*, 1912, **12**, 527.
[32] S. Dagley and C. N. Hinshelwood, *J.*, 1938, 1930.
[33] R. M. Lodge and C. N. Hinshelwood, *J.*, 1939, 1683.
[34] E. A. Poole and C. N. Hinshelwood, *J.*, 1940, 1565.

it is attained.[30] Lag may be specifically affected without change in total population.[15] Aeration of the medium may affect total population but not necessarily growth rate.[35, 29]

(d) *The Phase of Decline.*—Unless they are renewed by division, cells die, the death rate being increased by antiseptics, high temperatures, and various radiations. According to some observers, the number of survivors at time t out of an initial population of N_0 is given by the exponential decay law, $N = N_0e^{-lt}$, where l is a constant (curve I in Fig. 8). Others maintain that the true form of curve is more like that of II in the figure, and that I is found only as an approximation observed over a certain range, and especially when some of the cells have already died before the first measurements are made (as would be found if observation began at the point a on curve II). The experimental decision of the question whether the exponential curve is indeed the ideal form seems to be not quite easy.[36] An important theoretical question lies at the basis of the matter. If the death of a cell exposed to an unfavourable environment occurred after a definite time, the number of survivors would vary with time as in curve III : if the population were non-homogeneous initially, III would be rounded off to give IV, which, by assuming the appropriate frequency distribution of resistance could be adjusted to reproduce II. A very special distribution could even change the curve into I, but such a distribution would be improbable, since it would involve a maximum proportion of cells, not with the average resistance, but with the very lowest resistance. If we accept the (controversial) fact that I may in certain examples be accurately followed, and if we reject as improbable the assumption of the very special initial distribution necessary to explain it in terms of the varying resistance theory, then some very interesting conclusions follow. The differential equation of I is $-1/N \cdot dN/dt =$ constant, *i.e.*, the probability of death for any individual in any given element of time is independent of the previous history, as in radioactive decay. If death is caused by the presence of an antiseptic (for example), yet is independent of the time of exposure, it can only mean that at some moment a chance conjunction of events occurs exposing the cells to lethal influences which have not so far affected them. For the analogous action of X-rays on the cells of *Bact. coli*, it has been suggested that the chance event is an encounter of a quantum of radiation with some localised sensitive site in the cell.[37] This is a special hypothesis which leads to a result of the correct form, but the possible implications of the exponential law are of a more general character.

2. *Cell Division and Cell Morphology.*

Another phenomenon which seems to depend upon conjunctions of events is the division of the growing cell. Cells do not all attain the same size

[35] O. Rahn and G. L. Richardson, *J. Bact.*, 1942, **44**, 321.

[36] A. J. Clark, "General Pharmacology"; cf. R. C. Jordan and S. E. Jacobs, *J. Hyg.*, 1944, **43**, 275.

[37] D. E. Lea, R. B. Haines, and C. A. Coulson, *Proc. Roy. Soc.*, 1936, *B*, **120**, 47; 1937, **123**, 1; J. A. Crowther, *ibid.*, 1926, *B*, **100**, 390.

before dividing : nor are the successive intervals of time between divisions of individual cells precisely equal. They are distributed about a mean in an apparently random fashion. In experiments on *Bact. lactis ærogenes* at 30°, 30% of the divisions occurred after intervals between 30 and 35 minutes, and 91% of the total occurred within the range 20—50 minutes. The distribution was not far from symmetrical about a mean of 30—35 minutes. Very few cells had a fission time of more than double the mean. The variation was, however, striking : it was not due to inhomogeneity of the strain, for daughter cells from an early division were not consistently different from the average.[38]

Under certain conditions, divisions may be so much delayed that very long filamentous or snake-like cells are formed.[39, 40, 41] In these conditions the size distribution becomes much more scattered than normal. The factors conducive to long-cell formation with scattered size distribution are (*a*) the presence of certain drugs which inhibit division without inhibiting growth to the same extent, and (*b*) the transfer of the cells to an unaccustomed growth medium, to which the growth and division functions adapt themselves at different rates.[42, 43, 44]

With one coliform organism the distribution of cell sizes at a given moment has been found to follow approximately the law $n(l) = e^{-l/\bar{l}}$, where $n(l)$ is the number of cells of length greater than l, and $\bar{l}$ is the mean length. This suggests that the attainment of a given size greater than the mean is governed by a conjunction of probabilities statistically analogous to that which permits a molecule to traverse a free path greater by an assigned factor than the mean. The view has been put forward, on the basis of various experiments on *Bact. lactis ærogenes*, that two independent factors control division and elongation of the cell (the latter being diffusible into the medium) and that these two factors are separately modifiable by adaptation.[42]

3. *The Influence of Drugs on Bacterial Growth.*

The influence of drugs on bacteria is varied : some act as general protoplasmic poisons, some by interfering with specific members of the series of enzyme reactions upon which growth depends. Some have specific effects on lag, mean generation time, or on cell division probability. According to the evidence brought forward by Fildes and others,[45, 46, 47, 48, 49, 50, 51]

[38] G. D. Kelly and O. Rahn, *J. Bact.*, 1932, **23**, 147.
[39] E. W. Ainley Walker and W. Murray, *Brit. Med. J.*, 1904, **2**, 16.
[40] R. Tunnicliff, *J. Infect. Dis.*, 1939, **64**, 59.
[41] A. D. Gardner, *Nature*, 1940, **146**, 837.
[42] C. N. Hinshelwood and R. M. Lodge, *Proc. Roy. Soc.*, 1944, *B*, **132**, 47.
[43] R. M. Lodge and C. Hinshelwood, *Trans. Faraday Soc.*, 1943, **39**, 420.
[44] G. H. Spray and R. M. Lodge, *ibid.*, p. 425.
[45] P. Fildes, *Brit. J. Exp. Path.*, 1940, **21**, 315.
[46] D. D. Woods, *ibid.*, p. 74.
[47] H. McIlwain, *ibid.*, p. 136.
[48] H. McIlwain, *ibid.*, p. 148.
[49] P. Fildes, *ibid.*, 1941, **22**, 293.
[50] H. McIlwain, *Biochem. J.*, 1942, **36**, 417.
[51] G. P. Gladstone, *Brit. J. Exp. Path.*, 1939, **20**, 189.

specific inhibitory actions are often exerted by substances which are structurally related to normal metabolites of the cell. Their structure makes them liable to be taken up by the enzymes in competition with the normal substrate, for which, however, they are in other respects not adequate substitutes. Competing pairs of substances in this sense are thiol compounds and mercury,[45] aminobenzoic acid and sulphonamides,[46] nicotinic acid and pyridine-3-sulphonic acid,[48] amino-acids and their sulphonic acid analogues,[48] and so on.

Only a few aspects of the extensive subject of drug action can be dealt with here.

(a) *Influence of Drug Concentration on Antibacterial Action.*—Much of the earlier work,[5, 36] consisted of measurements on the rate at which disinfectants killed cells, the death rate being expressed in the form ac^n, where a is constant and n is frequently a quite high power of the concentration c of the drug. This power law could hardly have a real theoretical significance and would seem to be an approximation for a law of rather different form. Suppose a certain concentration, c_0, of the drug could be tolerated by the cell, being dealt with by neutralising mechanisms of some kind : the death rate might well be proportional to $c - c_0$. Then we have :

$$\log \text{rate} = \log \text{const.} + \log (c - c_0)$$

and

$$\mathrm{d}(\log \text{rate})/\mathrm{d}c = 1/(c - c_0)$$

If we also write rate $= ac^n$, then $\mathrm{d}(\log \text{rate})/\mathrm{d}c = n/c$, and comparison of the two expressions gives $n = c/(c - c_0)$. If the tolerance is appreciable, $c - c_0$ will be small over the range in which the first serious action of the drug is exerted, *i.e.*, n will be large, though it would not appear constant over more than a limited range. The tolerance to small concentrations which this view implies is in fact observed in some cases. In the action of bacteriostatic agents such as proflavine (2 : 8-diaminoacridine sulphate) on the lag of coliform organisms, the initial tolerance is not only observable but is to be expected theoretically. Fig. 9 shows results found for proflavine and *Bact. lactis ærogenes*.[52] Each curve of the family corresponds to a strain of cells which has been repeatedly grown in presence of a certain concentration of the drug (see later). The form of the curves is explained as follows : the proflavine interferes with the working of an enzyme which yields an intermediate constituting the substrate of a second enzyme. The rate of working of this second enzyme is related to the concentration of the intermediate by an adsorption isotherm (Fig. 10). Normally, the concentration of intermediate prevailing in the cell has a value such as A_1; it can be reduced to B before any inhibition is manifest, *i.e.*, there will be a tolerance proportional to A_1B, after which further drug causes a fall in rate to, say, C. The cells which have been trained in presence of drug at higher concentrations have greater reserves of the intermediate-forming enzyme, so that the concentration

[52] D. S. Davies, C. N. Hinshelwood, and J. M. G. Pryce, *Trans. Faraday Soc.*, 1945, in press.

of the intermediate itself starts at values $A_2, A_3 \ldots$., with correspondingly greater tolerances. If the curves in Fig. 9 were expressed in terms of a law making the lag depend upon the nth power of the drug concentration, then the values of n would have to be high. Actually, the curves can be represented by a formula derived rationally from the above theory.

The influence of various inhibitors on the mean generation time can sometimes be expressed quite well by a simple linear relation of growth rate and concentration : $R = R_0(1 - bc)$, where R is the growth rate in presence of a concentration c of drug, R_0 that with none, and b is a constant.[53]

(b) *Drug Action and Structure.*—This subject cannot be dealt with in detail but reference should be made to a few general matters. Two effects

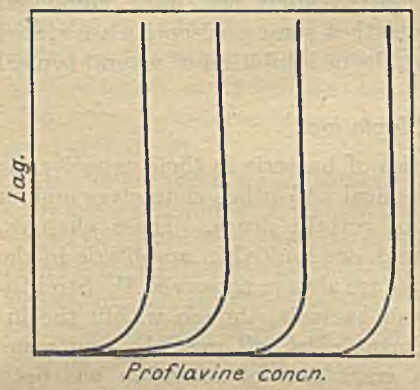


Fig. 9.

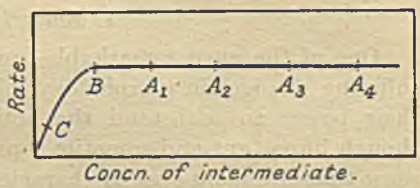


Fig. 10.

of structure have to be distinguished. In the first place, the partition of the drug between the medium and the part of the cell where it acts will depend upon the structure of the drug molecule. In particular, it will show regular variations during the ascent of a homologous series.[54] Many quite large quantitative differences in drug action are explained away when varying phase distributions are allowed for, *e.g.*, by comparing actions at equivalent chemical potentials.[55] The antibacterial action of various phenols seems to be related to the solubility of the phenol in olive oil (a possible model for certain regions of the interior of the cell).[56] The inhibitory action of straight-chain aliphatic alcohols increases by a constant factor from one member of the homologous series to the next higher : this shows that each CH_2 group has its own contribution to make to the total effect, and indicates that the action occurs in a part of the cell capable of attaching alcohol to its substance by each link of the hydrocarbon chain.[53, 54, 57, 58]

The differences in antibacterial action of such classes of compound

[53] S. Dagley and C. N. Hinshelwood, *J.*, 1938, 1942.
[54] K. H. Meyer, *Trans. Faraday Soc.*, 1937, **33**, 1062.
[55] J. Ferguson, *Proc. Roy. Soc.*, 1939, *B*, **127**, 387.
[56] A. H. Fogg and R. M. Lodge, *Trans. Faraday Soc.*, 1945, in press.
[57] F. W. Tilley and J. M. Schaffer, *J. Bact.*, 1926, **12**, 303.
[58] W. S. Stiles, " Introduction to Plant Physiology," 1936, p. 81.

as sulphonamides, acridine derivatives, triphenylmethane dyes, and so on can hardly be explained in this way. The actions depend upon the intervention of the drug at specific stages of the reactions involved in growth. The antibacterial action of substances related to known metabolites, already referred to, supports this view, for which further evidence is provided by the phenomenon of "cross training". For example, *Bact. lactis ærogenes* trained to resist proflavine has also increased resistance to methylene-blue, but not to sulphonamide. When thoroughly trained to sulphanilamide, it will resist sulphaguanidine and *vice versa*: but sulphonamide training does not immunise to proflavine. This offers yet another method of classifying specific inhibitory actions. On the other hand, with *Staphylococcus aureus* nicotinamide antagonises not only sulphapyridine but quite unrelated drugs too.[59] It is also of interest to note that some antiseptics are stated to cause death of cells before causing significant inhibition of several typical enzymes of those cells.[60]

4. *Bacterial Adaptation.*

One of the most remarkable properties of bacteria is their capacity for suffering changes in morphology, biochemical and other characters and in their power to withstand the action of certain drugs. These changes, though important and sometimes profound, are limited in amplitude in the sense that the main species characteristics are always preserved.[61] Streptococci are never transformed into coliform bacteria, though within the individual groups continuous ranges of forms exist, all possibly, and some demonstrably, interconvertible.[62–66] A great deal of attention has been paid to the changes in the forms of colony in which bacteria grow on solid media. One initial type often gives rise to variants, sometimes stable, sometimes unstable,[67, 68] the changed colony form being linked in varying degrees with other changes [69–74] in character. This phenomenon is often, though not very happily, referred to as bacterial dissociation. The adaptation of bacteria to utilise new food sources has already been mentioned. There is no reason to suppose that the development of the power to resist the action of certain drugs is other than a special example of the operation of the general adaptive mechanism.

To illustrate some of the principles involved in adaptation we will con-

[59] W. B. Wood and R. Austrian, *J. Exp. Med.*, 1942, **75**, 383.
[60] M. A. Bucca, *J. Bact.*, 1943, **46**, 151.
[61] A. I. Virtanen, *ibid.*, 1934, **28**, 447.
[62] P. Fildes, *Brit. J. Exp. Path.*, 1927, **8**, 219.
[63] L. W. Parr, *Bact. Rev.*, 1939, **3**, 1.
[64] C. Nyberg, K. Bonsdorff, and K. Kauppi, *Z. Bakt.*, 1937, **139**, 13.
[65] O. Sievers, *ibid.*, p. 27.
[66] F. Neri, *ibid.*, 1940, **146**, 166.
[67] P. Hadley, *J. Infect. Dis.*, 1937, **60**, 129.
[68] F. Faragó, *Z. Bakt.*, 1934, **133**, 139.
[69] H. B. Gillespie and L. F. Rettger, *J. Bact.*, 1939, **38**, 41.
[70] C. S. Flynn and L. F. Rettger, *ibid.*, 1934, **28**, 1.
[71] M. I. Bunting, *ibid.*, 1940, **40**, 57, 69; 1942, **43**, 593.
[72] I. M. Lewis, *ibid.*, 1934, **28**, 619.
[73] M. W. Deskowitz, *ibid.*, 1937, **33**, 349.
[74] L. W. Parr and M. L. Robbins, *ibid.*, 1942, **43**, 66.

sider a particular example in more detail. *Bact. lactis ærogenes* grown in presence of proflavine acquires an immunity to the drug, the relation between lag and test concentration for strains trained at a series of concentrations being shown in Fig. 9.[75] The differences in the spacing of the curves correspond almost exactly to the training concentration,[76] showing that the training consists in the graded response of the cell to the actual inhibition, rather than in the selection of an inherently resistant strain from a mixed population of resistant and non-resistant cells.

(The Reporter [77] has discussed some of the literature bearing on the question of adaptation versus selection. There seems to be good evidence that adaptation is usually *initiated* by actual changes in individual cells. Naturally, when some of the cells have become adapted they will outgrow any unadapted cells, and in this sense selection must always be superposed on any other adaptive mechanism. But it is the initiation of the process which is of greater interest from the physicochemical point of view. Very varied opinions have been expressed about the subject in general.[67, 78–81])

Under favourable conditions the adaptation is very rapid, being complete after a few cell divisions, though it does not occur during the lag phase itself, *i.e.*, in the absence of any actual increase of cell substance. For the system under consideration the following simple model accounts for many of the facts. We assume an enzyme I whose total substance expands according to the equation $dx_1/dt = k_1x_1$. It gives an active intermediate which is partly used by another enzyme, II, and partly lost by diffusion. The intermediate attains in the region between the two enzymes a concentration c, such that $dc/dt = k'_1x_1 - k_2cn - k_3cx_2/(1 + bc) = 0$. The term k_2cn is the total rate of loss by diffusion and is thus proportional to the *total* area of cell wall, *i.e.*, to n, the number of cells. The total substance of enzyme II expands at a rate related to c by a Langmuir isotherm, so that the consumption of c from this cause is given by the term $k_3cx_2/(1 + bc)$. Assuming that cell division waits until the amount of enzyme II per cell attains to some standard amount, *i.e.*, that $n = \beta x_2$, we can easily solve the equations. The ratio x_1/x_2 tends on growth of the cells to a definite limit, whatever its initial value. If now a drug impairs the production of the intermediate so as to slow down the synthesis of enzyme II, the overall multiplication rate will be lowered. The ratio x_1/x_2 will, however, expand as growth occurs, and c will rise, this in turn causing an increase in the rate of growth of enzyme II until the multiplication rate returns to normal. The whole process constitutes an adaptation to the drug. There is, in fact, evidence that the enzymic

[75] D. S. Davies, C. N. Hinshelwood, and J. M. G. Pryce, *Trans. Faraday Soc.*, 1944, **40**, 397.

[76] *Idem, ibid.*, 1945, in press.

[77] See ref. (25).

[78] E. W. Todd, *Brit. J. Exp. Path.*, 1930, **11**, 368.

[79] R. R. Mellon, *J. Bact.*, 1942, **44**, 1.

[80] A. C. Giese, *ibid.*, 1943, **46**, 323.

[81] D. S. Davies and C. N. Hinshelwood, *Trans. Faraday Soc.*, 1943, **39**, 431.

constitution of drug-resistant bacteria has been changed in various ways.[82–85]

In the light of the hypothesis that adaptation consists in a change of enzyme balance resulting directly from the modification of the relative reaction rates of different cell processes, it is possible to discuss the spontaneous loss of training which sometimes occurs on subsequent growth in the drug-free medium, the marked retention of the training which occurs in other cases, the induced reversion of trained strains caused by growth in presence of other antibacterial agents, and similar problems.

The above-mentioned model provides a reasonable expression for the lag–concentration relation. If we assume that the increase of lag caused by the proflavine is essentially due to the lowered rate of working of an enzyme such as enzyme II, this lowered rate being caused by a reduced concentration of the active intermediate from its normal value c'_1 to a new value c, then we have the following equations :

$c = c'_1 - \phi(m)$, $\phi(m)$ being some function of the drug concentration.

$L = A/R$, where L is the lag and R the rate of working of the enzyme, and A a constant :

$$R = kc/(1 + bc).$$

From these we obtain

$$1/(L - L_0) = k/A[c'_1{}^2/\phi(m) - c'_1]$$

With proflavine, the experimental results are well represented with $\phi(m) = fm$, where f is a constant, corresponding to a virtually quantitative titration. The value of c'_1 for the various trained strains proves to be related to the training concentration P by the linear equation $c'_1 = \text{const.}\ (54 + P)$. The effect of training is thus to restore c from the reduced value it has initially in presence of the drug to the original value it would have had for untrained cells in the absence of any drug at all. Cells trained to methylene-blue also show increased resistance to proflavine. The lag–concentration curves for methylene-blue are of a very markedly different shape, indicating a different form for the function $\phi(m)$. If the form of the latter is calculated from the experimental results for untrained cells in presence of methylene-blue, then it can be used to predict how methylene-blue-trained cells should behave both at other methylene-blue concentrations and in presence of proflavine. Certain outstanding qualitative differences are correctly accounted for, and there is even a measure of quantitative agreement.[86]

No more is to be claimed for the views just outlined than that they illustrate the general sort of way in which adaptation may be related to

[82] W. P. Wiggert and C. H. Werkman, *Biochem. J.*, 1939, **33**, 1061.

[83] R. A. McKinney and R. R. Mellon, *J. Infect. Dis.*, 1941, **68**, 233.

[84] M. Landy, N. W. Larkum, E. J. Oswald, and F. Streightoff, *Science*, 1943, **97**, 295.

[85] E. H. Rennebaum, *J. Bact.*, 1935, **30**, 625.

[86] J. M. G. Pryce, D. S. Davies, and C. N. Hinshelwood, *Trans. Faraday Soc.*, 1945, in press.

kinetic principles. For more general accounts of adaptive enzymes, and in particular for the distinction which may be drawn between adaptive and constitutive enzymes, reference should be made to the discussions of J. Yudkin [87] and of R. Dubos.[88]

In contrast to hypotheses about the progressive modification during adaptive changes of the enzyme balance of cells stand those views which refer adaptation and variation to alternative possibilities presented to the cell at the moment of division.[89, 90] Such views would account naturally for cases where variant forms are found to be produced in a stable ratio,[71, 72, 73] and also for completely irreversible variations (which are believed to occur, though the evidence for complete irreversibility must be negative only).[67, 90]

Not very much is known about the nuclear apparatus of bacteria, or about the way in which the cell proteins are organised. If there is some sort of centre of organisation, and if an abnormal mode of division occurs, it is quite conceivable that appreciable modification of the actual protein patterns is produced in the new cells. This might well lead to rather important changes in properties. Perhaps, therefore, we might imagine that simple changes in the quantitative efficiency with which a given carbon or nitrogen source is utilised or in the resistance to a given drug can be explained by nothing more profound than the automatic shift in enzyme balance of the kind just discussed. On the other hand, to explain rather more profound changes in qualitative character we must perhaps invoke changes in the protein pattern dependent upon nuclear changes. The equation on p. 16 provides a basis for the expansion of precisely those enzymes which are needed in the utilisation of a given foodstuff, so that shifts in enzyme balance are the natural explanation of adaptive changes to new media.[26, 42, 87, 88, 91]

Spontaneous variations in bacteria occur: more frequent and more profound changes are caused by exposure to radiations such as *X*-rays.[92] These changes probably involve activation energies (or local entropy decreases) very unlikely to be provided under the normal conditions of life. Still more profound changes in the fundamental patterns, of the kind which would distinguish one species from another, would involve activation energies so high that they would only be available under conditions which would usually kill the cells. C. N. H.

3. Zeolites as Absorbents and Molecular Sieves.

Certain minerals show remarkable occlusive properties, and because of the way in which they have influenced current views of sorption a survey of these properties is opportune. Of all mineral occlusives, none are so well

[87] *Biol. Rev.*, 1938, **13**, 93.
[88] *Bact. Rev.*, 1940, **4**, 1.
[89] G. R. Reed, *J. Bact.*, 1933, **25**, 545.
[90] Cf. discussion by A. Haddow, *Acta Int. Union against Cancer*, 1937, **2**, 376.
[91] Cf. G. S. Mirick, *J. Exp. Med.*, 1943, **78**, 255.
[92] Cf. the discussion in E. Schroedinger, "What is Life?", 1944.

known as the zeolites.[1] These crystalline minerals (Table I) should be distinguished from amorphous gel zeolites and permutites used in water softening. They are aluminosilicates $(R,R_2')O,Al_2O_3,nSiO_2,mH_2O$, where R = Ca, Sr, and Ba and R′ = Na, K. The ratio of base to Al_2O_3 is always 1 : 1 and the ratio (Al + Si) : O = 1 : 2. Although the minerals are widespread, massive deposits are not usually found. They are formed under comparatively alkaline and probably stagnant conditions by the hydrothermal alteration of older rocks and lavas at temperatures believed to lie as a rule between 100° and 350°.[2] Under more acid conditions hydrothermal reactions would largely yield clays.[3] Zeolites, like clays, are frequently

TABLE I.

Some Typical Zeolites.

Zeolite group and zeolite.*	Probable ideal formula.	*X*-Ray studies, ref. no.	Crystal chemical nature.	Approx. interstitial volume available to H_2O (c.c./g.).
Mordenite group.				
Mordenite	$(Ca,K_2,Na_2)Al_2Si_{10}O_{24},6\frac{1}{2}H_2O$	—	Robust three-dimensional network[7]	0·135
Ptilolite	$Ca,K_2Na_2)Al_2Si_{10}O_{24},4H_2O$	—	—	0·102
Heulandite group.				
Heulandite	$CaAl_2Si_4O_{12},5H_2O$	8, 8*a*	Laminar structure	0·148
Brewsterite	$(Sr,Ca,Ba)Al_2Si_6O_{16},5H_2O$	—	—	0·136
Epistilbite	Like heulandite	—	—	0·155
Philipsite group.				
Phillipsite	$(K_2,Ca)Al_2Si_4O_{12},4\frac{1}{2}H_2O$	9	—	0·165
Harmotome	$(K_2,Ba)Al_2Si_{12}O_{32},5H_2O$	10	Robust three-dimensional network[11]	0·141
Stilbite	$(Na_2,Ca)Al_2Si_6O_{16},6H_2O$	—	—	0·172
Gismondite	$CaAl_2Si_2O_8,4H_2O$	—	—	0·206
Laumontite	$CaAl_2Si_4O_{12},4H_2O$	—	—	0·153
Chabazite group.				
Chabazite	$(Ca,Na_2)Al_2Si_4O_{12},6H_2O$	8	Robust three-dimensional network	0·214
Gmelinite	$(Na_2,Ca)Al_2Si_4O_{12},6H_2O$	—	Robust three-dimensional network[7]	0·211
Levynite	$CaAl_2Si_4O_{12},5H_2O$	—	—	0·184
Faujasite	$(Na_2,Ca)Al_2Si_4O_{12},10H_2O$	—	—	0·281
Natrolite group.				
Natrolite	$Na_2Al_2Si_3O_{10},2H_2O$	12*a*, 12*b*, 12*c*	Chain structures with a rather weak cross-linking of aluminosilicate chains[12]	0·095
Scolecite	$CaAl_2Si_3O_{10},3H_2O$			0·138
Mesolite	Between scolecite and natrolite			0·095—0·138
Thomsonite	$NaCa_2Al_5Si_5O_{20},6H_2O$			0·140
Edingtonite	$BaAl_2Si_3O_{10},3H_2O$			0·11
Analcite.	$NaAlSi_2O_6,1H_2O$	14	Robust three-dimensional network	0·078

* The classification is a modification of that given by C. Dana,[4] but the grouping may still need revision as more *X*-ray and crystal chemical data accrue. The crystal chemical classification in col. 4 is based in part on *X*-ray data and in part on studies of the stability of the zeolite as an absorbent and towards heating.[5] Chemical formulæ are ideal values, and isomorphous replacements of the type Ca ⇌ 2Na or CaAl ⇌ NaSi may cause considerable modification of these formulæ.[6]

[1] Earlier data on sorption equilibria in zeolites are discussed by J. W. McBain, "Sorption of Gases by Solids," Routledge, 1932, Chap. 5. Some kinetic aspects are summarised by Barrer, "Diffusion in and through Solids," Cambridge Univ. Press, 1941, Chap. 3.

[2] Cf. Lindgren, "Mineral Deposits," McGraw Hill Book Co., 1919, p. 427.

[3] F. Norton, *Amer. Min.*, 1939, **24**, 1; 1941, **26**, 1. A survey of work on hydrothermal formation of minerals, including zeolites and clays, is available up to 1937 (G. Morey and C. Ingerson, *J. Econ. Geol.*, 1937, **32**, 607—761).

[4] "A System of Mineralogy," 6th Edtn.; see also W. Bragg, "Atomic Structure of Minerals," Oxford Univ. Press, 1937, Chap. 16.

[5] W. Milligan and H. Weiser, *J. Physical Chem.*, 1937, **41**, 1029; R. M. Barrer, *Proc. Roy. Soc.*, 1938, *A*, **167**, 392, 406.

highly hydrated (Table I), and they have a rather low density and refractive index which reflect this high water content. They have a well-developed property of base-exchange, which is, however, shared by other minerals which are not zeolites such as the sodalite–hauyne group of minerals,[15] ultramarine, and some clays.

Like all silicates, zeolites are built by the union of SiO_4'''' tetrahedra by sharing one or more oxygen atoms with neighbouring tetrahedra. Some SiO_4'''' tetrahedra are replaced by AlO_4''''' tetrahedra, thus imparting a negative charge to the framework, which is neutralised by an electrochemical equivalent of interstitial cations. The framework is always sufficiently open to enmesh also interstitial water molecules, and upon this property depends their behaviour as occlusives. As indicated in Table I, three kinds of anionic framework exist : [14] (i) Robust three-dimensional aluminosilicate network structures. (ii) Structures with aluminosilicate sheets rather weakly bonded to one another. (iii) Structures with rather weakly cross-linked aluminosilicate chains.

The zeolites lose their crystal water on heating, with a variable degree of lattice shrinkage which is a minimum with the first kind of zeolite, and may be large, or even amount to irreversible lattice collapse, in the case of the laminar or fibrous zeolites.[5] From the present point of view the non-collapsing zeolites are the most important, for these not only regain their crystal water readily, but may sometimes occlude other gases and vapours in place of water.* The water or other molecules are situated in the same

[6] Cf. M. Hey, *Min. Mag.*, 1932, **23**, 51; A. Winchell, *Amer. Min.*, 1925, **10**, 90; 1926, **11**, 82.

[7] R. M. Barrer, *Trans. Faraday Soc.*, 1944, **40**, 555.

[8] J. Wyart, *Bull. Soc. franç. Min.*, 1933, **56**, 81; see also [8a] W. H. Taylor, *Proc. Roy. Soc.*, 1934, *A*, **145**, 80.

[9] J. Wyart and P. Chatelain, *Bull. Soc. franç. Min.*, 1938, **61**, 121.

[10] J. Sekawina and J. Wyart, *ibid.*, 1937, **60**, 139.

[11] R. M. Barrer, unpublished data on the sorptive properties of harmotome.

[12a] W. H. Taylor, C. Meek, and W. Jackson, *Z. Krist.*, 1933, **84**, 373; [12b] W. H. Taylor and R. Jackson, *ibid.*, 1933, **86**, 53; [12c] M. Hey and F. Bannister, *Min. Mag.*, 1932, **23**, 51, 243, 305, 421, 483.

[13] W. H. Taylor, ref. (8*a*). [14] W. H. Taylor, *Z. Krist.*, 1930, **74**, 1.

* Chabazite,[1] gmelinite,[7] mordenite [7] and "active" analcite,[16] all robust network structures (Table I), absorb non-polar gases, although not all robust network structures will do so, and the property depends on the relative dimension of molecule and interstitial zeolitic channel. Laminar and fibrous zeolites, if not over-heated during outgassing, may occlude small polar molecules such as ammonia in place of water, but have not yet been found to occlude non-polar gases.[17] G. Friedel [18] reported that harmotome could absorb hydrogen sulphide and ethyl alcohol in place of water, but for the latter at least this report requires reinvestigation.[11] In view of recent work on sorption by fibrous zeolites,[5] it is also most unlikely that the fibrous zeolite thomsonite could occlude glycol and *iso*propyl alcohol, as reported by M. Hey.[19] In these cases effects noted with larger organic molecules could have been due to selective removal of water from the liquid. M. Grandjean [20] stated that levynite, gismondite, and harmotome could occlude mercury vapour. This is possible even for harmotome in view of the small radius of the mercury atom (~1·5 A.).

Molecules which may replace water as constituents of the lattices of a number of zeolites are given in Table V.

interstices as the interstitial cations, with which they are in close association.[13, 7] Like these cations they may diffuse from one site to another, but whereas cations cannot leave the lattice unless their place is taken by an electrochemical equivalent of other cations (base-exchange) the water and other molecules may do so, and thus form vagabondising constituents of the crystal lattice. In this they recall the behaviour of hydrogen in solution in palladium, cerium, thorium, and other metals.[7, 21] The gas–zeolite system is best considered as an interstitial solid solution in which the occluded molecule interacts physically only with its environment, through dispersion, polarisation, and ion-dipole forces.[21] The term "persorption" applied to gas–zeolite systems is inadequate and should be reserved for the *irregular* dispersion of the sorbate in the sorbent, as is the case in charcoal or silica gel.

As should characterise binary interstitial solid solutions in which the occluded component is a vapour, most zeolites give a continuous sorption–desorption isotherm (Fig. 1). This property is also reported [22] for water in a number of other crystalline substances, including $MgPt(CN)_6$, strychnine sulphate, basic zirconium oxalate, oxalates of cerium and some rare earths, some flavanol derivatives, glucosides, and crystalline proteins. In addition, certain clays and other silicates,[23] such as siloxene,[24] will take up water or ammonia continuously, probably within the crystal lattice, or between individual aluminosilicate sheets.[25]

Isotherms, Isobars, and Isosteres.—Typical isotherms, isobars, and isosteres in zeolites are illustrated in Figs. 1, 2, and 3. A number of equations have been suggested to describe the isothermals.[26] The most successful of these are based upon kinetic or statistical models [27] and have the form

$$p = \text{constant} \times \theta/(1 - \theta) \quad . \quad . \quad . \quad . \quad . \quad (1)$$

[15] See W. H. Bragg, ref. (4), p. 265, for structural details of the hauyne–sodalite group.

[16] R. M. Barrer and D. A. Ibbitson, *Trans. Faraday Soc.*, 1944, **40**, 195, 206.

[17] R. M. Barrer, ref. (5).

[18] *Bull. Soc. franç. Min.*, 1896, **19**, 94.

[19] *Min. Mag.*, 1932, **23**, 103.

[20] *Bull. Soc. franç. Min.*, 1910, **33**, 5.

[21] R. M. Barrer, *Trans. Faraday Soc.*, 1944, **40**, 374.

[22] A list of references to work on crystals with zeolitic components, and crystals giving continuous sorption isothermals is given by McBain, ref. (1).

[23] J. Sameshima and N. Morita, *Bull. Chem. Soc. Japan*, 1935, **10**, 485, 490; I. Cornet, *J. Chem. Physics*, 1943, **11**, 1; L. Hansen, W. Samuel, and P. Forni, *Ind. Eng. Chem.*, 1940, **32**, 116; L. Spitze and L. Hansen, *ibid.*, 1942, **34**, 506.

[24] Kautsky and his collaborators have studied the sorptive properties of siloxene. A list of references is given by McBain, ref. (1). See also H. Kautsky and F. Grieff, *Z. anorg. Chem.*, 1938, **236**, 124.

[25] G. Nagelschmidt, *Z. Krist.*, 1936, **93**, 481; see also S. B. Hendricks, *J. Physical Chem.*, 1941, **45**, 65.

[26] O. Weigel, *Sitz. Ges. Naturwiss. Marburg*, 1924, 73; G. Huttig, *Fortschr. Chem.*, 1924, **18**, 5; *Kolloid-Z.*, 1924, **35**, 337; O. Schmidt, *Z. physikal. Chem.*, 1928, *A*, **133**, 263; E. Rabinowitsch, *ibid.*, 1932, *B*, **16**, 43.

[27] M. Hey, *Min. Mag.*, 1935, **24**, 99; E. Rabinowitsch and W. C. Wood, *Trans. Faraday Soc.*, 1936, **32**, 947; R. M. Barrer, ref. (21).

where θ denotes the fraction of all the available interstices occupied by solute molecules, and p is the equilibrium pressure. Values for the constant in

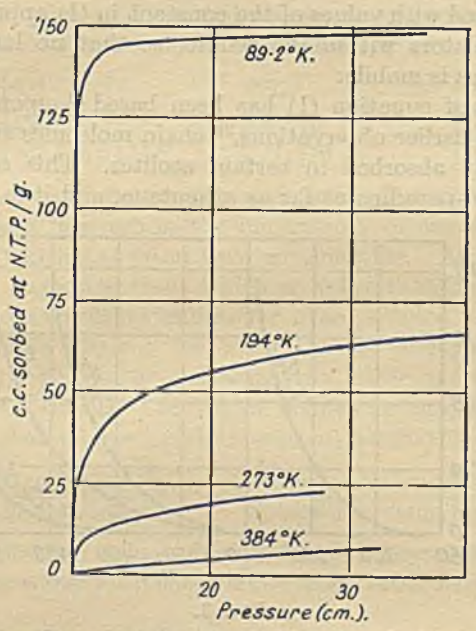


FIG. 1.

Isotherms of N_2 *in chabazite.*[17]

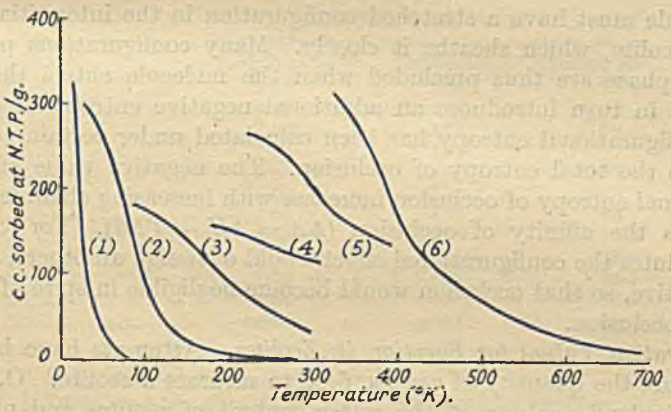


FIG. 2.

Isobars of typical gases in chabazite.[30]

Curve 1 : He (400 *mm.*).
Curve 2 : H_2 (400 *mm.*).
Curve 3 : N_2 (400 *mm.*).
Curve 4 : CO_2 (200 *mm.*).
Curve 5 : NH_3 (100 *mm.*).
Curve 6 : H_2O (7 *mm.*).

(1) have recently been obtained[21] corresponding to the progressive conversion of translational and rotational degrees of freedom of the solute molecule into vibrational ones when the solute is transferred from the gas phase to

the zeolitic interstice. The value of this constant is a sensitive measure of the mobility of the solute within the crystal lattice, and actual isotherms are best reproduced with values of the constant in (1) appropriate for three-dimensional oscillators within the zeolite, so that no large proportion of occluded molecules is mobile.

An extension of equation (1) has been based [21] upon the discovery [16] that, contrary to earlier observations,[28] chain molecules such as *n*-paraffins may be copiously absorbed in certain zeolites. This sorption has been followed for the *n*-paraffins as far as *n*-heptane, and it is certain that such

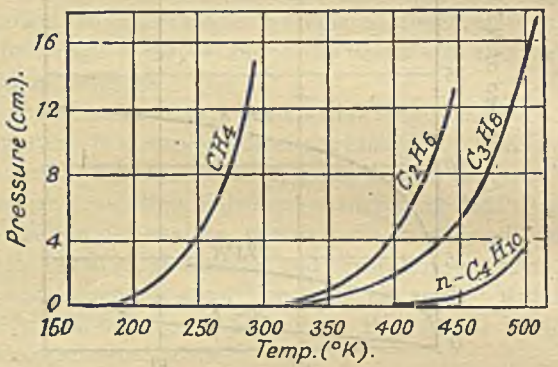


FIG. 3.

Isosteres for some hydrocarbons occluded in chabazite. The amount sorbed is 12·18 *c.c./g. of mineral.*[16]

a molecule must have a stretched configuration in the interstitial channel of the zeolite, which sheaths it closely. Many configurations possible in the gas phase are thus precluded when the molecule enters the crystal, and this in turn introduces an additional negative entropy of occlusion. The configurational entropy has been calculated under certain conditions, and also the total entropy of occlusion. The negative value of the configurational entropy of occlusion increases with increasing chain length, and decreases the affinity of occlusion ($\Delta A = \Delta U - T\Delta S$). For very long-chain solutes the configurational effect would outweigh all others, and make ΔA positive, so that occlusion would become negligible in spite of the great heat of occlusion.

Saturation Values for Sorption in Zeolites.—Attempts have been made to measure the quantity of gas required to saturate a zeolite. O. Weigel [29] summarised all analyses of the water content of zeolites and plotted the amount of water, in order of increasing value, against the number of analyses. The curves obtained had lengthy central portions either nearly flat or of gentle slope, suggesting a fairly definite if not quite sharp saturation value towards water, because at room temperature water is very

[28] O. Schmidt, ref. (26); T. Baba, *Bull. Chem. Soc. Japan*, 1930, **5**, 190; J. Sameshima, *ibid.*, 1929, **4**, 96.

[29] *Centr. Min.*, 1922, 164—178, 201—208.

strongly absorbed, and occlusion isotherms are nearly rectangular. The water contents in Table I may thus be taken as substantially saturation values towards this species. Reported approximate saturation values for other vapour–chabazite systems, in c.c. (at N.T.P.)/g., are :

He	400 [30]	NH_3	270,[30] 170 [17]
H_2	350,[30] 280 [17]	CO_2	170 [30]
A	250 [17]	$CH_3{\cdot}OH$	165 [31]
N_2	175,[30] 170,[30] 164 [17]	$C_2H_5{\cdot}OH$	110 [31]

These data are not always concordant, and the lack of uniformity is probably in large degree a measure of the uncertainty of the extrapolation of experimental data, which has often been considerable. Another procedure has therefore been suggested [16] in which a well-established experimental figure is taken, and saturation values for other solutes derived from it. Satisfactory figures are 170 c.c. (at N.T.P.)/g. for nitrogen–chabazite, or 266 c.c. (at N.T.P.)/g. for water–chabazite, the latter figure being derived from the formula of Table I. Long-chain solutes are stretched out within the narrow zeolitic channel (see previous section), so that

$$\frac{\text{Length (or diameter) of } N_2 \text{ (or } H_2O)}{\text{Length of chain molecule}} = \frac{\text{Saturation value for chain molecule}}{\text{Saturation value for } N_2 \text{ (or } H_2O)}.$$

In this way the saturation values given in Table II were obtained. Saturation values towards water may be obtained from Table I for other zeolites,

TABLE II.

Saturation Values in Chabazite and Active Analcite.

Gas.	Diameter or length (A.).	Saturation value, c.c. (at N.T.P.)/g. Chabazite.	Active analcite.
H_2O	2·76	266 *	97 *
NH_3	3·60	193	72
H_2	3·74	186	69
A	3·84	181	67
O_2	3·83	181	67
N_2	4·08	170 †	63
CH_4	4·00	173	64
C_2H_6	5·54	125	46·4
C_3H_8	6·52	106	39·5
n-C_4H_{10}	7·78	89	33·1
n-C_5H_{12}	9·04	77	28·5
n-C_6H_{14}	10·34	67	25·0
n-C_7H_{16}	11·56	59	22·2

* Experimental value from Table I.

† Value used to calculate all others save that for water.

but molecular sieve effects (p. 44) or lattice collapse on dehydration (see next section) may effectively prevent other gases from replacing the water.

Phase Formation and Zeolitic Solid Solution.—A recent investigation has combined *X*-ray measurements with dehydration isobars,[32] and suggests

[30] E. Rabinowitsch, ref. (26); E. Rabinowitsch and W. Wood, ref. (27).

[31] O. Weigel and E. Steinhoff, *Z. Krist.*, 1925, **61**, 125.

[32] W. Milligan and H. Weiser, ref. (5).

that, among fibrous zeolites, scolecite and natrolite give evidence of lower hydrates but that thomsonite and mesolite dehydrate without discontinuity, as do stilbite and heulandite (laminar structures) and chabazite and analcite (robust three-dimensional networks). The less robust chain and sheet structures show lattice changes during heating which may extend to complete breakdown of the parent structure; but the chabazite and analcite lattices show little change in *X*-ray pattern on heating. The existence of an ammoniate of natrolite has also been demonstrated; [17] above a threshold pressure, sorption of ammonia occurred autocatalytically to give an approximately univariant system. It has been suggested that zeolitic solid

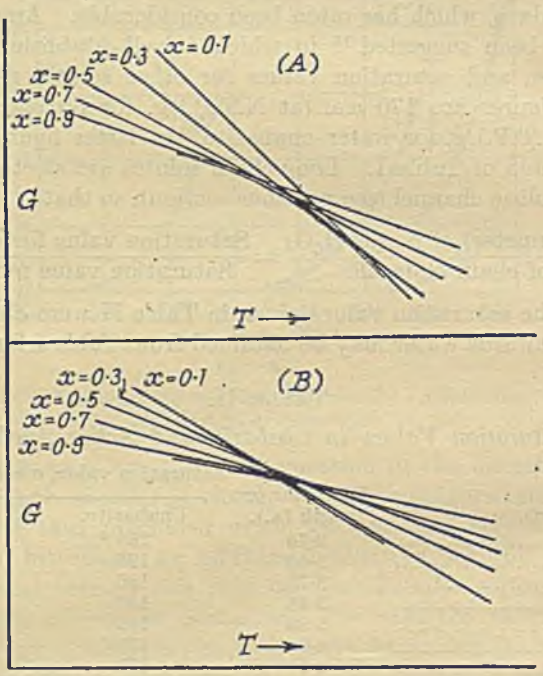


FIG. 4.

Gibbs free energy (G) *plotted against* T *for the system Zeolite,* $x\mathrm{NH_3} + (1-x)\mathrm{NH_3}$. *Fig.* 4A *shows the condition for a continuous sorption isobar, and Fig.* 4B *the condition for a discontinuous sorption isobar,* x *changing at a certain temperature from* 0·9 *to* 0·1.[21]

solution occurs normally when the zeolitic lattice suffers no marked change during sorption or desorption, and that new phases tend to form if concomitant lattice changes are considerable.[17] Another criterion has been suggested which shows how the alternatives of zeolitic solid solutions (ammonia–chabazite) or phase changes (ammonia–natrolite) may arise. In a system Zeolite, $x\mathrm{NH_3} + (1-x)\mathrm{NH_3}$, for example, when the free energy–temperature curves lie for different values of x as in Fig. 4A, the sorption–desorption isobar is continuous, but with a small displacement as in Fig. 4B, one or more discontinuities may arise.[21] This is because the lower envelope, representing the most stable state of minimum free energy, curves con-

tinuously and touches each G–T curve at one point only in Fig. 4A; but in Fig. 4B this envelope consists of two intersecting lines, coinciding with the G–T curves for $x = 0{\cdot}9$ and $x = 0{\cdot}1$.

Heats and Free Energies of Zeolitic Solid Solution.—Heats of occlusion in zeolites have been measured directly,[33] and by the use of the Clapeyron equation.[34] Although it is not certain that the calorimetric and thermodynamic methods measure exactly the same heat, these heats do not differ significantly where both methods have been used on the same sample.[35] For non-polar gases these heats are within experimental accuracy independent of temperature, but are characteristic functions of the amount of gas occluded [17, 16, 21] (Fig. 5). A rather similar form of curve is also

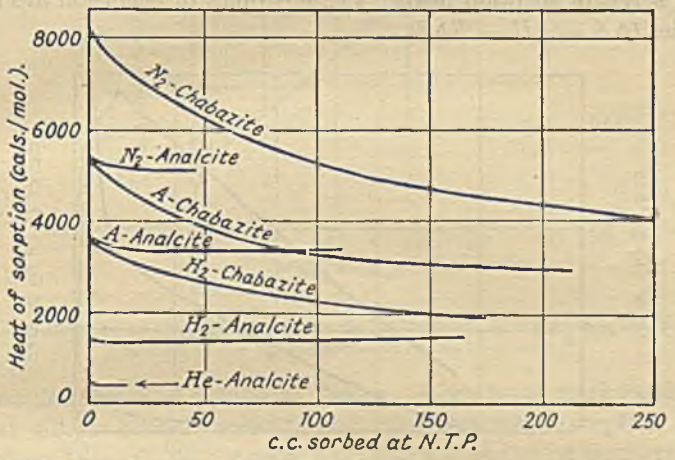


FIG. 5.

Heats of sorption of N_2, H_2, He *and* A (*without theats of compression*) *on* 3·98 *g. of chabazite and* 7·86 *g. of active analcite respectively, as a function of the amount of gas occluded.*[17]

obtained for van der Waals adsorption on glass,[36] charcoal,[37] graphite,[38] and simple ionic crystals,[39] and various causes of it have been discussed.[38, 17, 39] Sorption heats in chabazite, even of small permanent gases such as nitrogen, are unusually large,[17] and it has been shown [40] that this can be due to the interstitial position of the solute molecule. For equal diameters of solute and interstice the van der Waals energy of interaction may amount to about 8 times the value observed when the occluded molecule is transferred

[33] A. Lamb and E. Ohl, *J. Amer. Chem. Soc.*, 1935, **57**, 2154; M. G. Evans, *Proc. Roy. Soc.*, 1931, *A*, **134**, 97; M. Hey and F. Bannister, *Min. Mag.*, 1934, **23**, 483.

[34] *Idem*, ref. (12); M. Hey, ref. (27); A. Tiselius, *Z. physikal. Chem.*, 1935, *A*, **174**, 401; E. Rabinowitsch, ref. (26); R. M. Barrer, refs. (5) and (7).

[35] *E.g.*, M. Hey, ref. (27); see also S. Brunauer, "The Adsorption of Gases and Vapours," Vol. 1, Oxford Univ. Press, 1944, Chap. 8.

[36] W. Keesom and J. Schweers, *Physica*, 1941, **8**, 1007, 1020, 1032.

[37] A. van Itterbeek and W. van Dingenen, *ibid.*, 1937, **4**, 389.

[38] R. M. Barrer, *Proc. Roy. Soc.*, 1937, *A*, **161**, 476.

[39] W. J. Orr, *ibid.*, 1939, *A*, **173**, 349.

[40] J. de Boer and J. Custers, *Z. physikal. Chem.*, 1934, *B*, **25**, 225.

to a plane surface of the same sorbent. The sorption heat of *n*-paraffins in chabazite is nearly a linear function of the number of CH_2 groups in the molecule,[16] and rises by about 3000 cals. per CH_2. For high molecular weight *n*-paraffins the sorption heat could become greater than for many chemical reactions, although it is due only to dispersion and polarisation forces.[16]

Entropies (p. 36) and free energies of occlusion have also been obtained for sorption in chabazite and active analcite, as functions of the charge of gas and at different temperatures.[16] Free energy–0 curves have the same form as the corresponding heat of sorption–0 curves, and the standard free energy series for a given zeolite and group of solutes parallels the corresponding series of sorption heats; *i.e.*, entropies of occlusion are all of the same order ($\Delta A = \Delta U - T\Delta S$).

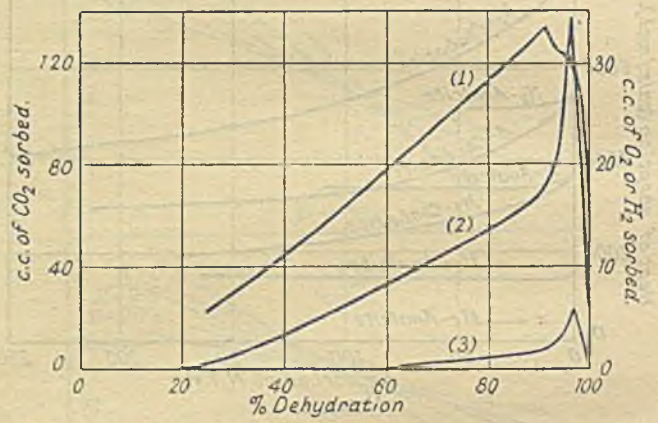


FIG. 6.

Sorption at 0° *and* 1600 *mm. of* CO_2, O_2, *and* H_2 *by chabazite at various degrees of dehydration.*[41]

Curve (1) : CO_2. *Curve* (2) : O_2. *Curve* (3) : H_2.

Factors Influencing Sorption Equilibrium in Zeolites.—The power of a zeolite to occlude depends in large measure upon the severity and duration of the heating and degassing treatment to which it has been subjected. These determine the extent of both lattice collapse or alteration and of dehydration. Fig. 6 shows the equilibrium sorption of carbon dioxide by chabazite as a function of the water content of the mineral.[41] A maximum occurs at about 95% dehydration; conditions severe enough to remove more water probably lead simultaneously to lattice collapse. Parallel with this striking result, the heat of occlusion was measured as a function of the degree of hydration [42] (Fig. 7).

Sorption equilibrium may also be modified by base-exchange. A recent study using mordenites revealed that base-exchange may alter both mole-

[41] A. Lamb and J. Woodhouse, *J. Amer. Chem. Soc.*, 1936, **58**, 2637.
[42] A. Lamb and E. Ohl, ref. (33).

cular sieve properties of zeolites (p. 44) and sorption equilibria and heats.[7] Affinities and heats of sorption of nitrogen and oxygen increased, for the exchanging ions $Na^+ < Ba^{++} < Ca^{++}$, in the order of the relative polarising power of the interstitial cation. Similar results were deduced [7] from the relative escaping tendency of water vapour from base-exchange stilbites, chabazites, and heulandites.[43]

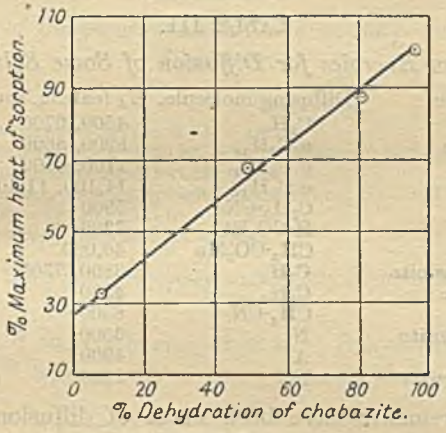


FIG. 7.

Heats of sorption of $CH_3 \cdot OH$ *in chabazite (at a charge of* 0·000178 *mol./g.) as a function of the % dehydration of the chabazite.*[33]

Kinetic Behaviour of Solutes in Zeolites.—One must distinguish between the usual simple zeolitic solution and the less usual sorption in which a new phase appears, with discontinuity in the isobar or isotherm (p. 38). In the latter case the sorption rate was " autocatalytic " and the process was regarded as a growth and diffusion of phase boundaries.[17] When simple zeolitic solutions are formed, occlusion has been interpreted as a pure diffusion process not complicated by slow rates of transfer of solute across the ingoing surface. As a diffusion process, sorption rates follow Fick's laws, and the " parabolic " diffusion equation has been most successfully employed.[44, 7, 16] Potential solutes may be divided into three groups (see Table V) : (*a*) molecules occluded extremely rapidly, (*b*) those occluded slowly at room temperature or above, (*c*) those excluded from the zeolite lattice. These groups grade continuously into one another. Group (*a*) comprises small molecules, or molecules of small cross section (*e.g.*, in chabazite, gmelinite, and active analcite, helium, hydrogen, oxygen, nitrogen, argon, methane, and ethane; and in natural mordenite, helium, hydrogen, oxygen, and nitrogen). For the first three minerals, Group (*b*) included *n*-paraffins, and Group (*c*) included *iso*-paraffins and aromatic hydrocarbons, but in mordenite Group (*b*) included methane and ethane, and Group (*c*) *n*-paraffins. Group (*b*) solutes enter the lattice by a diffusion

[43] E. Lowenstein, *Z. anorg. Chem.*, 1909, **63**, 69.
[44] P. Emmett and T. DeWitt, *J. Amer. Chem. Soc.*, 1943, **65**, 1253.

process of which each unit act requires an energy of activation.[16, 7, 44] This energy is needed to move the solute molecule from one position of maximum sorption potential to another within the crystal, and in an appropriate lattice was observed even for the inert gas argon. Table III records a number of apparent energies of activation, which for the *n*-paraffin series increase somewhat with increasing charge of gas and chain length.

TABLE III.

Apparent Activation Energies for Diffusion of Some Solutes into Zeolites.[45]

Zeolite.	Diffusing molecule.	E_A (cals./g.-mol. of solute).
	C_3H_8	4500, 6700
Chabazite	n-C_4H_{10}	8900, 8600
	n-C_5H_{12}	7100, 6800
	n-C_7H_{16}	11,100, 11,400, 9600
	C_2H_5·CN	5900
	H·CO_2Et	7300
	CH_3·CO_2Me	40,000
Active analcite	C_3H_8	6800, 7300
Mordenite	C_2H_6	4300
	CH_3·CN	8300
Ba–Mordenite	N_2	3500
	A	4900
Ca–Mordenite	N_2	1040

Several measurements have been made of diffusion constants, D, of ammonia and water in analcite and heulandite.[46] In heulandite, diffusion anisotropy occurs, and at 20° normal to the (201) and (001) planes (each normal to the unit lamellæ of the heulandite layer lattice), $D_{001} : D_{201} = 1 : 11{\cdot}6$—20. In one sample the activation energies for the diffusion across (001) and (201) planes were 5400 and 9140 cals./g.-mol. A kinetic theory of the diffusion constant has been successfully applied to Tiselius's measurements of D.[47] The diffusion constant is a function of the charge of solute already within the lattice. At first, inhomogeneity of the zeolite lattice, chemical or physical, causes solute molecules to be anchored at sites of highest sorption potential, so restricting their mobility. Again, when nearly all sites are occupied the chance of an adjacent vacant site into which the solute might move is small and so mobility is again reduced. In a *homogeneous* zeolite, D is related to θ, the fraction of the interstices occupied, and D_0, its value when $\theta = 0$, by $D = D_0 (1 - \theta)$.[48] A considerable volume of evidence has been obtained that D does behave in the manner predicted.[16, 7, 1]

Rates of Occlusion of Solutes as a Function of Water Content, Heat Treatment, and Grain Size.—The treatment accorded to a zeolite may greatly modify the sorption rates of solutes. One important variable is the extent of dehydration, the mineral occluding gases more rapidly the more completely the water is removed, providing conditions do not simultaneously

[45] R. M. Barrer, *J. Soc. Chem. Ind.*, in press.

[46] A. Tiselius and S. Brohult, *Z. physikal. Chem.*, 1934, *A*, **168**, 248; A. Tiselius, *ibid.*, 1934, *A*, **169**, 425; *idem*, ref. (34).

[47] M. Hey, ref. (26); see also *idem*, *Phil. Mag.*, 1936, **22**, 492.

[48] R. M. Barrer, ref. (1); *Trans. Faraday Soc.*, 1941, **37**, 590.

give lattice collapse. Conditions for producing very active chabazite in small amount have been established,[16] and the influence of degree of dehydration on some sorption rates and equilibria measured [44] (cf. p. 40). Molecular sieve properties of chabazite in relation to its water content are summarised in Table IV. These results were extended by the observation

TABLE IV.

Effect of Degree of Dehydration upon Sorption Rates and Equilibria in Chabazite.

Dehydration, %.	Gas.	Behaviour.
50	CO_2	Considerable sorption at −78°.
	N_2	Appreciable sorption at −78°; occlusion at negligible rate at −195°.
	H_2	Considerable sorption at −195°.
67	N_2	Still only small sorption in finite time at −183°.
	O_2	Copiously occluded at −183°.
	A	Copiously occluded at −183°.
96	N_2, O_2, A	All three gases copiously and rapidly occluded at −183°.

that nitrogen entered the chabazite when 67% dehydrated by a process of activated diffusion,[44] whereas there is no measurable energy of activation

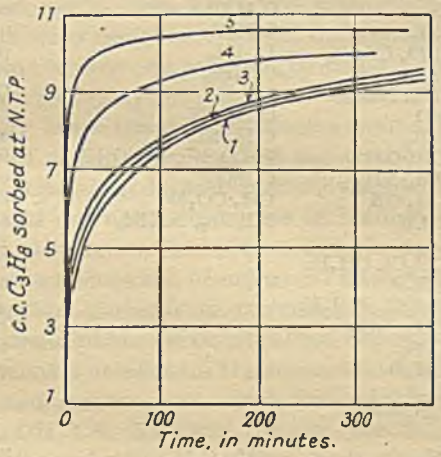


FIG. 8.

Influence of state of subdivision of chabazite upon the rate of occlusion of propane in it. In all experiments, the mass of chabazite = 3·336 g., the initial pressure of $C_3H_8 = 9{\cdot}6 \pm 0{\cdot}1$ *cm.* Hg *and* $T = 200°$ C.[16]

Curve 1 : *Particles* $\frac{1}{8}$—$\frac{3}{16}$ *inch diameter, outgassed* 14 *hrs. at* 470—480° C.
Curve 2 : *Particles* 20—30 *mesh, outgassed* 14 *hrs. at* 470—480° C.
Curve 3 : *Particles* 80—100 *mesh, outgassed* 14 *hrs. at* 470—480° C.
Curve 4 : *Particles* < 200 *mesh, outgassed* 14 *hrs. at* 470—480° C.
Curve 5 : *Particles* 180—200 *mesh, outgassed* 7 *hrs. at* 470—480° C.

when this gas enters a well outgassed chabazite.[17] It seems likely that water molecules immobilised in the zeolitic channel at −183° impede the flow of nitrogen within the crystal. Other examples in which one substance

impedes the sorption of a second substance by a zeolite have been reported, and may in some cases be due to the same cause.[45]

An investigation of the influence of degree of subdivision upon sorption rates of *n*-paraffins revealed that, in qualitative accord with the theory of diffusion, the smaller the particle dimensions the more rapid was the sorption [16] (Fig. 8).

Molecular Sieve Properties.—The sorptive properties of several zeolites have now been extensively investigated, and in Table V are summarised

TABLE V.

Molecules Occluded or Excluded by the Three Classes of Molecular Sieve.[45]

	Typical molecules rapidly occluded at room temp. or below.	Typical molecules moderately rapidly or slowly occluded at room temp. or above in the thermal stability range.	Typical molecules occluded at negligible rates, or totally excluded within the thermal stability range.
Section (i).			
Class 1 minerals	He Ne A H_2 N_2 CO CO_2 COS, CS_2 H_2O HCl, HBr NO NH_3 $CH_3{\cdot}OH$ $CH_3{\cdot}NH_2$ $CH_3{\cdot}CN$ HCN Cl_2 CH_3Cl, CH_3Br CH_3F CH_2Cl_2, CH_2F_2 CH_4, C_2H_6 C_2H_2 CH_2O H_2S $CH_3{\cdot}SH$	C_3H_8 and simple higher *n*-paraffins $C_2H_5{\cdot}OH$ $C_2H_5{\cdot}NH_2$ C_2H_5F C_2H_5Cl C_2H_5Br I_2, HI CH_2Br_2 CH_3I $C_2H_5{\cdot}CN$ $C_2H_5{\cdot}SH$ $H{\cdot}CO_2Me$, $H{\cdot}CO_2Et$ $COMe_2$ $CH_3{\cdot}CO_2Me$ $NHMe_2$, $NHEt_2$	Branched-chain hydrocarbons. *cyclo*-Paraffins. Aromatic hydrocarbons. Derivatives of all these hydrocarbons. Heterocyclic molecules (*e.g.*, thiophen, pyridine, pyrrole). $CHCl_3$, CCl_4, $CHCl{:}CCl_2$, $CH_3{\cdot}CHCl_2$, $CHCl_2{\cdot}CCl_3$, C_2Cl_6 and analogous bromo- and iodo-compounds. Secondary straight-chain alcohols, thiols, nitriles, and halides. Primary amines with NH_2 attached to a secondary C atom. Tertiary amines. Branched-chain ethers, thioethers, and secondary amines.
Section (ii).			
Class 2 minerals	He Ne A H_2 O_2 N_2 CO NH_3 H_2O	CH_4, C_2H_6 $CH_3{\cdot}OH$ $CH_3{\cdot}NH_2$ $CH_3{\cdot}CN$ CH_3Cl, CH_3F HCN CS_2 Cl_2	All classes of molecules in cols. 3 and 4 above.
Section (iii).			
Class 3 minerals	He Ne H_2 O_2 N_2 H_2O	A HCl NH_3	All molecules referred to in col. 4, section (ii). Also: CH_4, C_2H_6, $CH_3{\cdot}OH$, $CH_3{\cdot}SH$, $CH_3{\cdot}CN$, $CH_3{\cdot}NH_2$, CH_3Cl, CH_3F.

the sorptive and molecular sieve characteristics of three distinct categories of sieve.[45] Class 1 is represented by well-outgassed chabazite, gmelinite, active analcite, and a synthetic crystalline zeolitic mineral; class 2 by well-outgassed natural mordenite; and class 3 by well-outgassed calcium and barium mordenites produced hydrothermally at high temperatures. Convenient definitions of the three classes of molecular sieve were given as follows: Class 1 exclude *iso*-paraffins, occlude *n*-paraffins slowly, and methane, ethane, and molecules of smaller cross-section rapidly, at room temperature. Class 2 exclude *n*-paraffins (and molecules of similar and larger cross-section), occlude methane and ethane slowly, and nitrogen and molecules of similar and smaller cross-section rapidly, at room temperature. Class 3 show negligible occlusion of methane and ethane, and molecules of larger cross-section, but occlude nitrogen, oxygen, and molecules of smaller cross-section rapidly.

The three classes of crystal sieve have been used to resolve molecular mixtures, frequently with striking success. For example, by using chabazite it has been shown that simpler *n*-paraffins may be separated quantitatively from all *iso*-paraffins and aromatic hydrocarbons, and that C_1 and C_2 hydrocarbons may be removed from propane and higher hydrocarbons.[45, 49]

The molecular sieve method was extended to the separation of polar and polarisable molecules from admixture with other molecules.[45] One or more constituents were removed from 52 different typical liquid mixtures containing as many as five components, by using a class 1 zeolite; 17 such separations were effected by means of a class 2 zeolite; and 6 by class 3 zeolites. In many cases these separations are single representatives only of whole groups of separations, so that the method is of some generality and power. The separation depends primarily upon differences in molecular shape and size, and not upon differences in boiling point. In this way it may supplement distillation technique, *e.g.*, in resolution of azeotropic mixtures, many of which (some of industrial importance) can be separated. The rate of separation varies from extremely rapid to very slow, but provided the components of the mixture do not decompose on heating, rise in temperature frequently accelerates the separation rate many times.

Monosubstituted methanes in which the substituent groups are small, such as Cl, CH_3, OH, CN, NH_2 and the like, are very rapidly occluded by chabazite, and monosubstituted ethanes with similar substituent groups enter the lattice considerably more slowly (Table V). Both classes of solute may be quantitatively separated from molecules in col. 4, section (i), of Table V, and the monosubstituted methanes can also be partly or completely separated from similarly substituted ethanes. In a natural mordenite the monosubstituted methanes were slowly occluded (CH_3·OH, CH_3·NH_2, CH_3·CN and the like), but monosubstituted ethanes were excluded (Table V). Separations were therefore obtained of one- from two-carbon molecules. On the other hand, the class 3 occlusives, calcium and barium mordenite, did not occlude even molecules with one carbon atom, so that the separations

[49] R. M. Barrer, B.P. No. 548,905; U.S.P. No. 2,306,610.

obtained were of simple inorganic molecules (NH_3, H_2O, HCl) from organic gases and vapours.

R. M. B.

4. Crystallography.

i. *Introduction.*

Sections iii and iv of this Report aim at giving a reasonably complete account of the more important inorganic and organic structures which have been determined by *X*-ray or electron-diffraction methods during the year. In a few cases, however, only a bare reference is given; and it is possible that certain other work may have been missed entirely, because many of the publications are still rather inaccessible.

On the other hand, no attempt is made to cover the wider aspects of the subject in the form of a systematic Annual Report. Instead, the plan is to have certain special articles at longer intervals which will review various branches of the subject. This year Section ii gives a brief account of ambiguities which may occur in the analysis of diffraction patterns, a subject which is clearly of importance in all structural work. The large and growing amount of *X*-ray work on natural and synthetic fibre structures demands some special treatment, and this is given in Section v. Finally, we include a brief general article on the electron microscope in Section vi. The applications of this instrument to problems of chemical interest are steadily increasing. Ultimately we may expect some overlap between electron microscope methods and the more usual diffraction methods, in the analysis of complex molecular structures. With this possibility in mind, the present section has been included in this Report and is of an introductory nature.

A number of important topics are of necessity entirely omitted from this Report, but it is hoped that these may in turn be covered by further special articles in the future. Among these subjects are optical and magnetic properties, lattice vibrations and the physics of crystals in general, the structure of metals, alloys, and minerals, the investigation of surface problems by electron diffraction,[1] the structure of proteins and other complex substances not of the fibre type, the diffraction of *X*-rays by liquids [2] and amorphous substances, and general improvements in experimental and interpretative technique. The recent extensive work on the structure of diamonds of different types [3] may also merit a special review when the results become clearer.

The new structure determinations now reported (Sections iii and iv) contain a fairly large number of reasonably accurate bond-length determinations, notably for halogen derivatives. The whole question of the derivation of bond lengths from the standard covalent radii is now under-

[1] G. P. Thomson, *J. Inst. Metals*, 1943, **69**, 191 (lecture).

[2] N. S. Gingrich, *Rev. Mod. Physics*, 1943, **15**, 90 (review).

[3] (Sir) R. Robertson, J. J. Fox, and A. E. Martin, *Phil. Trans.*, 1943, *A*, **232**, 463; *Proc. Roy. Soc.*, 1936, *A*, **157**, 579; (Sir) C. V. Raman *et al.*, *Proc. Indian Acad. Sci.*, 1944, 189; G. D. Preston, *Nature*, 1945, **155**, 69; (Mrs.) K. Lonsdale, *ibid.*, p. 144.

going considerable revision. Following the new single-bond covalent radii for oxygen, nitrogen, and fluorine,[4] contractions from predicted values are now seen to be more numerous than was previously thought, and their cause is the subject of much discussion. In general, the effect on the distance of the polar character of the bond between unlike atoms is probably greater than was formerly realised. The new data now recorded should be of great value in any general rediscussion of bond distances. Amongst new structure determinations that of ammonium pentachlorozincate, $(NH_4)_3ZnCl_5$, is of interest as it reveals only a four-fold type of co-ordination, the structure consisting of a packing of NH_4^+, Cl^-, and $ZnCl_4^-$ ions, a situation somewhat reminiscent of the structures of phosphorus penta-chloride and -bromide.[5]

In the organic section, the structure of the carboxyl group has received considerable attention and the latest determinations make the two C–O distances almost as different as for pure single and pure double bonds. That is for non-associated acids; on association, interesting changes are detected and the distances become more nearly equal, although still remaining quite distinct. Finally, in the ion, complete equality is to be expected. Other new results indicate that the conjugating power of phenyl groups may be less than has hitherto been supposed, and in compounds like diphenyl there appears to be very little contraction in the length of the connecting link. The tendency for coplanarity of the rings is correspondingly small, and in the vapour the molecule of diphenyl is probably not coplanar. The crystal structure should be re-examined. The structure of the highly symmetrical coronene molecule has now been examined by crystal analysis and the preliminary results are in good agreement with molecular orbital calculations of the interatomic distances.

J. M. R.

ii. *Ambiguities in the Analysis of Diffraction Patterns.*

If a crystal structure is expressed as a continuous distribution of scattering matter a knowledge of the absolute values of the structure amplitudes is not sufficient to define the distribution. An infinite number of distinct distributions can clearly be obtained by attaching arbitrary values to the unknown phase constants associated with the observed amplitudes, and evaluating the corresponding Fourier series. In nearly all actual cases the fundamental assumption is therefore made that the structure is composed of atoms, of a kind and number indicated by elementary chemical analysis. The problem can then be reduced to finding what co-ordinates must be attached to a limited number of scattering points in order to explain the observed diffraction pattern.

In single-crystal analysis the number of observed and measurable *X*-ray reflections is always much greater than the number of atoms whose positions have to be discovered. In fact, it should generally be possible to make 50

[4] V. Schomaker and D. P. Stevenson, *J. Amer. Chem. Soc.*, 1941, **63**, 37; see *Ann. Reports*, 1943, **40**, 86.

[5] *Ibid.*, 1942, **39**, 101.

or more observations per atom without difficulty. It might then be thought that atomic positions which yielded perfect agreements between the measured and the calculated X-ray intensities throughout the whole range of observations would constitute a unique solution of the structural problem involved. That this is not necessarily the case was proved originally by L. Pauling and M. D. Shappell [1] for a special point of the space-group $T_h^7 - Ia3$, where positive and negative values of one parameter were found to yield the same X-ray intensities. A similar property is shown by the corresponding special point for the space-group $O_h^{10} - Ia3d$.[2] In general, any distinct distributions of points which possess the same vector distances will obviously

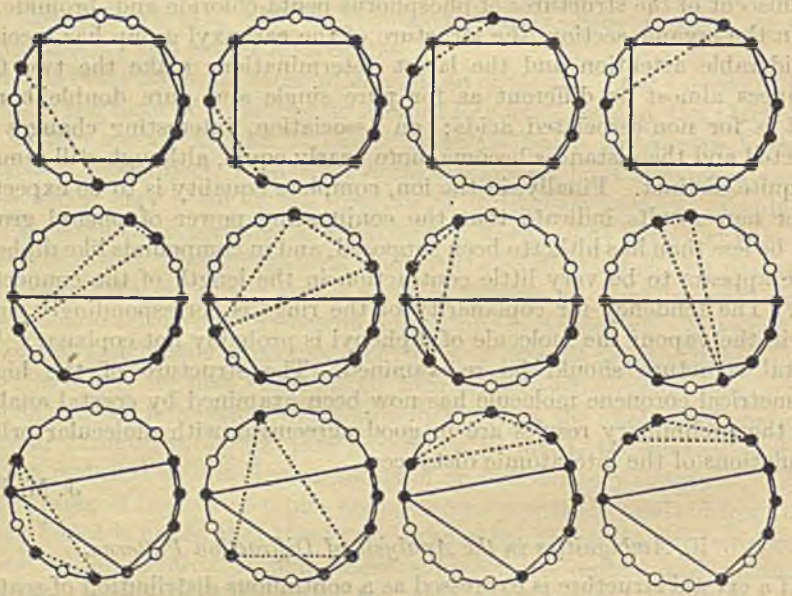


FIG. 1.

Three sets of homometric quadruplets for the cyclotomic set n = 16, r = 8 (A. L. Patterson, *Physical Rev.*, *loc. cit.*).

give rise to the same X-ray diffraction pattern. Such sets of points are called "homometric."

A. L. Patterson [3] has now provided a very useful general discussion of such ambiguities in X-ray crystal analysis. His treatment is confined chiefly to one-dimensional cases. Such linear periodic distributions can be conveniently represented by plotting the points on the circumference of a circle, and the cases examined are those obtained by using r of the n vertices of an inscribed regular polygon. Sets of points obtained in this way are called "cyclotomic" sets. Out of 2664 cyclotomic sets examined (up to $n = 16$) Patterson has found a total of 390 homometric pairs, 7 sets of homometric triplets, and 3 sets of quadruplets. The last distributions are shown

[1] *Z. Krist.*, 1930, **75**, 128. [2] A. L. Patterson, *Nature*, 1939, **143**, 939.
[3] *Physical Rev.*, 1944, **65**, 195.

by the black dots in Fig. 1. In these sets each member obviously represents a distinct distribution of points, yet all four would give rise to exactly the same *X*-ray diffraction pattern.

There appears to be considerable difficulty in developing any genera theory for the occurrence of homometric sets, but the treatment can be extended in various ways. For example, it is sometimes possible to introduce a variable parameter between certain points and so produce an infinity of homometric pairs. It is also possible to extend the treatment to two and three dimensions, but these possibilities have not yet been fully explored.

The results so far obtained, however, are very significant and they clearly reveal the limitations of the *X*-ray method of crystal analysis. The same type of ambiguity must also apply, in a more drastic manner, to the results of gas-diffraction experiments. There the molecules are widely separated and scatter independently. The experimental data give in effect the superposition of all the interatomic distances in the molecule, and as these distances are no longer vector quantities, the possibility of ambiguity must be greatly increased. For example, the cases illustrated (see Fig. 1) represent only linear periodic distributions for crystal analysis, but in gas-diffraction analysis cyclic molecules of these types would give rise to similar ambiguities.

These findings emphasise the fact that the results of diffraction experiments should in general be confirmed by other independent lines of physical and chemical evidence. Optical and magnetic properties, for example, afford valuable auxiliary evidence, and the various experiments should all agree before a structure is finally accepted as being true. The essence of a diffraction experiment is rather to show that a given postulated structure is consistent with the data, than to attempt to establish a truly unique solution. At the same time if alternative solutions should exist in any given case, it seems very unlikely that they would both be chemically reasonable.

The above discussion, of course, assumes complete ignorance of the phase constants of the *X*-ray reflections. If these can be determined, a truly unique solution to the problem immediately becomes possible. In a large and growing number of cases this can be done, for example, by the comparison of members of an isomorphous series, as in the phthalocyanines [4] or by successive approximations involving the use of a heavy atom in the molecule.[5] The ambiguities discussed above do not arise in such cases, and the results do represent unique solutions.

J. M. R.

iii. *Inorganic Structures.*

Halogen Derivatives of Tin, Arsenic, and Nitrogen.—A very extensive investigation by electron diffraction of 14 of these derivatives has now been reported by A. H. Skinner and L. E. Sutton.[1] From 6 to 9 plates were taken on the vapour of each substance, and these showed from 4 to 6 maxima for

[4] J. M. Robertson, *J.*, 1935, 615; 1936, 1195.

[5] *Idem*, *Nature*, 1939, **143**, 75; J. M. Robertson and (Miss) I. Woodward, *J.*, 1940, 36.

[1] *Trans. Faraday Soc.*, 1944, **40**, 164.

the various compounds. Both the radial distribution and the correlation method of analyses were used. The models employed for the tin compounds assumed the four valencies to be directed to the corners of a tetrahedron (not necessarily regular). For the compounds of nitrogen and tervalent arsenic non-coplanar valencies were assumed. Free rotation of the methyl group was assumed in all compounds, and tested with satisfactory results in the case of trimethyltin monochloride.

The final results regarding interatomic distances and valency angles are given in Table I, together with some previous results for the tetrahalides of tin and the trihalides of arsenic.[2] The table also gives the length of the bond to the halogen as calculated from the covalent radii of L. Pauling and M. L. Huggins; [3] for nitrogen, however, the new radius of 0·74 A. is used.[4]

The small but progressive contraction which occurs in the bond length when more halogens are added to the central atom is very clearly brought out by these results. (The percentage contraction from the calculated value is given in the last column of the table.) This phenomenon is a very general one and has already been investigated and discussed in other series of compounds, such as the fluorinated methanes [5] and the chlorinated silanes.[6]

TABLE I.

Compound.	Angle X–M–X.	C–M, A.	M–X, A. obs.	M–X, calc.	Contraction, %.
$SnMe_3Cl$	108°±4°	2·19±0·03	2·37±0·03	2·39	0·8
$SnMe_2Cl_2$	110 ±5	—	2·34±0·03	2·39	2·1
$SnMeCl_3$	108 ±4	2·19±0·05	2·32±0·03	2·39	2·9
$SnCl_4$	109·5	—	2·30±0·03	2·39	3·8
$SnMe_3Br$	~109·5	2·17±0·05	2·49±0·03	2·54	2·0
$SnMe_2Br_2$	109 ±3	~2·17	2·48±0·02	2·54	2·4
$SnMeBr_3$	109·5 ±2	~2·17	2·45±0·02	2·54	3·5
$SnBr_4$	109·5	—	2·44±0·02	2·54	3·9
$SnMe_3I$	~109·5	~2·17	2·72±0·03	2·73	0·4
$SnMe_2I_2$	109·5 ±3	—	2·69±0·03	2·73	1·5
$SnMeI_3$	109·5 ±2	—	2·68±0·02	2·73	1·8
SnI_4	109·5	—	2·64±0·02	2·73	3·3
$AsMe_2Cl$	98 ±3	—	2·18±0·04	2·20	0·9
$AsCl_3$	103 ±3	—	2·16±0·03	2·20	1·8
$AsMe_2Br$	96 ±3	—	2·34±0·04	2·35	0·0
$AsBr_3$	100 ±2	—	2·36±0·04	2·35	0·0
$AsMe_2I$	98 ±4	—	2·52±0·03	2·54	0·8
AsI_3	100 ±2	—	2·58±0·05	2·54	−1·6 *
NMe_2Cl	—	1·47±0·02	1·77±0·02	1·73	−2·3 *
$NMeCl_2$	108 ±1	—	1·74±0·02	1·73	−0·6 *

* Expansion.

As Skinner and Sutton point out, any straightforward explanation of this effect in terms of either bond multiplicity or ionic character of bonds is difficult, but the latter effect appears to be the more important for these

[2] L. O. Brockway and F. T. Wall, *J. Amer. Chem. Soc.*, 1934, **56**, 2373; M. W. Lister and L. E. Sutton, *Trans. Faraday Soc.*, 1941, **37**, 393, 406.

[3] *Z. Krist.*, 1934, **87**, 205.

[4] V. Schomaker and D. P. Stevenson, *J. Amer. Chem. Soc.*, 1941, **63**, 37.

[5] L. O. Brockway and H. O. Jenkins, *ibid.*, 1936, **58**, 2036.

[6] L. O. Brockway and I. E. Coop, *Trans. Faraday Soc.*, 1938, **34**, 1429.

cases. Each successive halogen addition will tend to increase the positive change on the central atom, with corresponding decrease of its normal radius. There will be a similar enlargement of the halogen atom with negative charge, but this effect on any one of the halogens will tend to diminish as more halogens are added. The net attractive force will also increase between the central atom and each halogen, and so from both causes we might expect a small progressive decrease in bond distance. We may expect a more quantitative development of this theory in the future.

Phosphoryl and Thiophosphoryl Halides.—Further interesting deviations from the predicted covalent bond radii for halogen–phosphorus, oxygen–phosphorus, and sulphur–phosphorus distances have been observed by J. H. Secrist and L. O. Brockway [7] in an electron-diffraction investigation of the compounds $POBr_3$, $PSBr_3$, $PSFBr_2$, and PSF_2Br. For the first two molecules trigonal symmetry is assumed and the structures are described in terms of two parameters, the Br–P–Br angle and the P–O or P–S : P–Br bond-length ratio. The calculations are therefore relatively straightforward. The lower symmetry of the other molecules makes the calculations more difficult, but a large number of models have been tested and examined. The radial distribution method has also been employed in each case.

The final results for the bond lengths (in A.) and valency angles in these molecules are given in Table II. Allowance being made for all uncertainties it is clear that the phosphorus–halogen distances (especially the P–F distance) are considerably shorter than the predicted values. The P–O and P–S distances are also distinctly less than the sum of the respective double-bond radii. In general, the new results confirm and extend the findings of previous investigations on similar compounds.[8]

TABLE II.

	$POBr_3$.	$PSBr_3$.	$PSFBr_2$.	PSF_2Br.	Covalent radius sum.
P–S	—	1·89±0·06	1·87±0·05	1·87±0·05	1·94 *
P–F	—	—	1·50±0·10	1·45±0·08	1·74
P–Br.....	2·06±0·03	2·13±0·03	2·18±0·03	2·14±0·04	2·24
P–O	1·41±0·07	—	—	—	1·57 *
$\widehat{BrPBr}$	108°±3°	106°±3°	100°±3°	—	—
$\widehat{FPBr}$	—	—	—	106°±3°	—

* Calculated for double bond.

Silicon Halides.—Further electron diffraction studies have been carried out by R. L. Livingstone and L. O. Brockway [8a] on silicon dimethyl dichloride, silicon methyl trichloride, and trifluorosilicon chloride. The Si–Cl distances are similar to previous measurements and the Si–C distances show some contraction, but details must be deferred.

[7] *J. Amer. Chem. Soc.*, 1944, **66**, 94.

[8] L. O. Brockway and J. Y. Beach, *ibid.*, 1938, **60**, 1836; D. P. Stevenson and H. Russell, *ibid.*, 1939, **61**, 3264; J. Y. Beach and D. P. Stevenson, *J. Chem. Physics*, 1938, **6**, 75.

[8a] *J. Amer. Chem. Soc.*, 1944, **66**, 94.

Silver Salts and the Ag–O *Bond.*—The crystal structure of potassium silver carbonate has been determined by J. Donohue and L. Helmholz.[9] The carbonate group is assumed to have the same configuration and dimensions as in calcite,[10] and the positions of the potassium and silver atoms can be determined with considerable accuracy from Fourier analyses of the visually estimated intensities. It is found that the silver atoms are surrounded by four oxygen atoms at 2·42 ± 0·05 A. There is one potassium–oxygen distance of 2·65 ± 0·08 A., and others varying from 2·9 to 3·0 A.

A brief report is also given [9] of the structure of silver carbonate, where the silver atoms are surrounded by deformed tetrahedra of oxygen atoms whose distance is estimated at about 2·3 A.

Silver oxalate has been examined by R. L. Griffith.[11] The monoclinic crystals appear to be rather similar to some other oxalates, with a short *b* translation (3·46 A.), and two molecules in the unit cell (space-group C_{2h}^5 — $P2_1/c$). Intensities were estimated visually, and Fourier projections serve to define the positions of the silver atoms with some accuracy, but the oxygen and the carbon atoms can hardly be located at all. Conclusions regarding the structure of the oxalate group are therefore unreliable, but these groups appear to be bound together to form chains by means of two short Ag–O bonds of length 2·17 and 2·30 A. Four other Ag–O distances range from 2·6 to 3·0 A.

The average Ag–O bond distance is found to vary considerably in different compounds,[12] from 2·51 A. in silver chlorate to 2·06 A. in the oxide. Such variations in the Ag–O distance are of interest in connection with the rule suggested by K. S. Pitzer and J. H. Hildebrand [13] which relates the colour of a compound formed from colourless ions to the amount of covalent character in the bond between these ions. For Ag–O the ionic radius sum is 2·46 A. and the covalent radius sum 2·19 A., so that for comparable structure types it should in some cases be possible to make estimates of the bond character. This matter is discussed in the paper by Donohue and Levine.[12]

Ammonium Pentachlorozincate.—A very full determination of the crystal structure of this salt, $(NH_4)_3ZnCl_5$, has now been made by H. P. Klug and L. Alexander.[14] The work is of interest in view of the rather infrequent occurrence of the MX_5 group in inorganic chemistry. In the examples which have already been studied the actual co-ordination has usually proved to be some combination of four- or six-fold types. In phosphorus pentachloride there is a combination of tetrahedral PCl_4^+ and octahedral PCl_6^- groups,[15] and in the pentabromide PBr_4^+ and Br^- ions.[16] The crystal

[9] *J. Amer. Chem. Soc.*, 1944, **66**, 295.
[10] N. Elliott, *ibid.*, 1937, **59**, 1380.
[11] *J. Chem. Physics*, 1943, **11**, 499.
[12] L. Helmholz and R. Levine, *J. Amer. Chem. Soc.*, 1942, **64**, 354.
[13] *Ibid.*, 1941, **63**, 2472.
[14] *Ibid.*, 1944, **66**, 1056.
[15] H. M. Powell, D. Clark, and A. F. Wells, *J.*, 1942, 642; *Ann. Reports*, 1942, **39**, 101.
[16] H. M. Powell and D. Clark, *Nature*, 1940, **145**, 971.

structures of Tl_2AlF_5 and K_2AlF_5,H_2O [17] show that infinite chains of AlF_6 octahedra extend through the crystal in such a way as to make the net composition AlF_5.

Ammonium pentachlorozincate crystals are orthorhombic, space-group D_{2h}^{16} — *Pnma*, and the unit cell contains four molecules of the composition $(NH_4)_3ZnCl_5$. The analysis was carried out on about 600 reflections (Cu-$K\alpha$ radiation) obtained from small crystals. The intensities were estimated visually from oscillation photographs, the multiple film technique [18] being employed. Analysis of the structure by trial proved too difficult, but the positions of the heavy atoms were finally obtained by evaluating the Patterson functions. Two-dimensional Fourier projections then gave the positions of all the atoms with considerable accuracy.

The unit cell is found to contain four tetrahedral $ZnCl_4$ groups, the average Zn–Cl distance being 2·25 ± 0·03 A., a value very close to the sum of the tetrahedral covalent radii [19] (2·30 A.). The remaining chlorine atoms are separated from the zinc atoms by over 4·4 A., and each of these chlorines is surrounded by a distorted octahedron of NH_4^+ ions, with an average $Cl–NH_4$ distance of 3·41 A., which is rather greater than the sum of the ionic radii. Each ammonium ion is in turn surrounded by groups of chlorine atoms.

The structure is thus seen to represent a packing of NH_4^+, Cl^-, and $ZnCl_4^=$ ions, which would be better expressed by writing the chemical formula as $(NH_4)_2ZnCl_4,NH_4Cl$. The Zn–Cl bonds are essentially covalent, but the other linkages are ionic (except N–H). Once again, therefore, the crystal is found to avoid the difficulty of five-fold co-ordination.

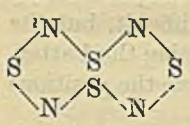
Sulphur Compounds.—C. S. Lu and J. Donohue [20] have examined a number of sulphur compounds by the method of electron diffraction. For sulphur itself they find that the S_8 molecule has essentially the same configuration as in the crystal,[21] *viz.*, a regular, puckered, eight-membered ring. It is interesting to note that several systematic distortions of this ring, such as the " tub," " chair," " cradle," and " butterfly " forms, were tested and shown to be incompatible with the radial distribution curve. Hence, the fraction of sulphur molecules which have these configurations in the vapour phase must be very small. However, the results do show that there must be a rather large thermal vibration associated with the puckered ring structure. The S–S distance of 2·07 ± 0·02 A., and the angle S–S–S of 105° ± 2°, are fairly close to previous determinations.

Orpiment sublimes at high temperatures apparently to give As_4S_6 molecules, for a model based on this structure [22] gives a satisfactory explanation of the pattern. In this model As–S = 2·25 ± 0·02 A., angle

[17] C. Brosset, *Z. anorg. Chem.*, 1937, **235**, 139.
[18] J. M. Robertson, *J. Sci. Instr.*, 1943, **20**, 175.
[19] L. Pauling and M. L. Huggins, *Z. Krist.*, 1934, **87**, 205.
[20] *J. Amer. Chem. Soc.*, 1944, **66**, 818.
[21] B. E. Warren and J. T. Burwell, *J. Chem. Physics*, 1935, **3**, 6.
[22] R. M. Bozorth, *J. Amer. Chem. Soc.*, 1923, **45**, 1621.

As–S–As = 100° ± 2°. These molecules also display rather large thermal vibrations.

Two other structures, those of sulphur nitride, S_4N_4, and realgar, As_4S_4, have been included in this electron-diffraction study, but although useful results have been obtained, which should assist in the exact analyses of the crystals, yet the structures cannot be established with certainty from the gas-diffraction data alone. However, several previously proposed structures can be definitely eliminated, and it is shown that an alternating eight-membered ring, of " cradle " shaped configuration, can lead to a satisfactory explanation of the diffraction pattern in both cases. The " cradle " model for sulphur nitride is rather closely related to the formula (inset) proposed by M. H. M. Arnold, J. A. C. Hagill, and J. M. Hutson,[23] which would involve resonance among several bond structures.



Other Structures.—Finally, reference may be made to a number of inorganic structures which have either been briefly reported, or for which the full papers are not available to the Reporter. These include magnesium carbide,[24] zinc cyanide,[25] cadmium iodide [26] (which has a random structure if crystallised quickly), and the compounds $SrMg_2$, $BaMg_2$, and $CaLi_2$.[27] The hydrate $SrCl_2,6H_2O$ appears to have been fully studied [28] and has a chain type of structure with linkages through the water molecules. Orthorhombic lead monoxide,[29] antimony trifluoride [30] (with an Sb–F distance of 2·0 A.), natrophilite, $NaMnPO_4$,[31] colemanite, $2CaO,3B_2O_3,5H_2O$,[32] ammonium chloroiridate,[33], sodium and ammonium iodate,[34] and the high-temperature modification of sodium nitrite,[35] have all received attention. The lattice constants of an isomorphous series magnesium, manganese, ferrous, cobalt, nickel, and zinc metantimonites, MSb_2O_4, have been measured, and the structures are reported to be similar to that of Pb_3O_4.[36] In another isomorphous series, comprising chromium vanadate and nickel, cobalt, copper, zinc, and cadmium chromates, both the metal atoms are surrounded by distorted octahedra or tetrahedra of oxygen atoms with common edges.[37]

J. M. R.

[23] *J.*, 1936, 1645.
[24] M. A. Bredig, *J. Amer. Chem. Soc.*, 1943, **65**, 1482.
[25] H. S. Shdanov, *Compt. rend. Acad. Sci. U.R.S.S.*, 1941, **31**, 352.
[26] G. Hägg and E. Hermansson, *Arkiv Kemi, Min., Geol.*, 1943, **17**, *B*, No. 10.
[27] E. Hellner and F. Laves, *Z. Krist.*, 1943, **105**, 134.
[28] A. T. Jensen, " 5 Nordische Kemikermode," 1939, 201.
[29] A. Byström, *Arkiv Kemi, Min., Geol.*, 1944, **17**, *B*, No. 8.
[30] A. Byström and A. Westgren, *ibid.*, 1943, **17**, *B*, No. 2.
[31] A Byström, *ibid.*, No. 4.
[32] V. A. Nikolsli, *Compt. rend. Acad. Sci. U.R.S.S.*, 1940, **28**, 59.
[33] G. B. Boki and P. I. Usikov, *ibid.*, 1940, **26**, 782.
[34] C. H. MacGillavry and C. L. Panthaleon van Eck, *Rec. Trav. chim.*, 1943, **62**, 729.
[35] B. Strijk and C. H. MacGillavry, *ibid.*, p. 705.
[36] S. Stahl, *Arkiv Kemi, Min., Geol.*, 1943, **17**, *B*, No. 5.
[37] K. Brandt, *ibid.*, 1943, **17**, *A*, No. 6.

iv. *Organic Structures.*

Ethylene.—An early study of ethylene [1] provided some single-crystal data, but the structure proposed is an improbable one because it fails to satisfy modern ideas regarding both interatomic and intermolecular distances. From the original data (which consist of only seven observed reflections) C. W. Bunn [2] has now calculated a reasonable structure, and has shown that a model with a C=C distance of 1·33 A. can give an even better account of the observed *X*-ray intensities than the structure originally proposed. In the new structure the closest approach between carbon atoms on neighbouring molecules is now 3·8 A., a reasonable value.

Structure of the Carboxyl Group.—The first detailed diffraction studies of the carboxyl group, carried out on the vapour of formic acid,[3] indicated that the carbon–oxygen distances were equal, at about 1·29 A. A later quantitative *X*-ray investigation of oxalic acid dihydrate crystals [4] gave slightly different distances, of 1·30 and 1·24 A. From an examination of the structures involved in the carboxyl group one would not expect equal distances in the case of the acids themselves. Later spectroscopic studies on the monomers and dimers of formic and acetic acids [5] have confirmed this view. Equality of the C–O distances might reasonably be expected, however, for the free carboxylate ion.

J. Karle and L. O. Brockway [6] have now published a comprehensive electron-diffraction study of the monomers and dimers of formic, acetic, and trifluoroacetic acids. The interesting question of what changes, if any, occur in the C–O distances on association does not appear to have been examined in detail before, and it is almost certainly beyond the reach of *X*-ray crystal analysis, where the crystal molecules would normally be associated in pairs, or in some larger groups, or with water molecules. In the gas-diffraction experiments the monomers were studied by photographing the equilibrium vapour at approximately 150°, which represents over 90%

TABLE III.

Carboxylic acids (distances in A.).

	Formic acid.		Acetic acid.		Trifluoroacetic acid.	
	Monomer.	Dimer.	Monomer.	Dimer.	Monomer.	Dimer.
C–OH	1·42±0·03	1·36±0·04	1·43±0·03	1·36±0·04	—	1·30±0·03
C=O	1·24±0·03	1·25±0·03	1·24±0·03	1·25±0·03		(average)
C–C	—	—	1·54±0·04	1·54±0·04	—	1·48±0·03
C–F	—	—	—	—	1·36±0·05	1·36±0·03
–OH....O=...	—	2·73±0·05	—	2·76±0·06	—	2·76±0·06
HO–C=O	117°±2°	121°±2°	122—138°	130°±3°	—	130°±3°

[1] W. H. Keesom and K. W. Taconis, *Physica*, 1935, **2**, 463.

[2] *Trans. Faraday Soc.*, 1944, **40**, 23.

[3] L. Pauling and L. O. Brockway, *Proc. Nat. Acad. Sci.*, 1934, **20**, 336, 340.

[4] J. M. Robertson and (Miss) I. Woodward, *J.*, 1936, 1817.

[5] L. G. Bonner and R. Hofstadter, *J. Chem. Physics*, 1938, **6**, 531; M. M. Davies and G. B. Sutherland, *ibid.*, p. 755.

[6] *J. Amer. Chem. Soc.*, 1944, **66**, 574.

monomer. For the experiments on the dimers, the vapour densities indicated a 9—15% dissociation, which could be allowed for in the calculations.

The results of the diffraction experiments concerning distances and angles are set out in Table III. For the monomers, *i.e.*, for the unassociated carboxyl group, a surprisingly large difference of nearly 0·2 A. is found between the two C–O distances. In fact, the longer distance (1·42 A.) is about the same as that observed in ether linkings, whereas the shorter corresponds to that found in ketones. The hydrogen must clearly be attached to one of the oxygens only, with the result that the carbon–oxygen bonds are more or less of the ordinary single- and double-bond types, respectively.

In the analysis of the dimers the molecules are assumed to have the planar centrosymmetrical type of bridge (inset), and the agreements obtained on this basis of calculation justify the assumption. With regard to the dimensions of the carboxyl group, it is significant that attempts to explain the diffraction pattern with models based on the monomer were unsuccessful. The final results (Table III) show a very considerable equalising of the C–O distances, brought about almost entirely by a shortening of the " single "-bond C–OH distance. However, there remains a considerable difference between the two C–O distances, about 0·1 A., which is in tolerable agreement with *X*-ray crystal results on other structures. Owing to this difference in C–O distances, it seems clear that the hydrogen atom must remain attached more strongly to one of the partners in the dimer than to the other. If it occupied an (average) centre position in the O–H . . . O bridge we would expect corresponding symmetry and equality in the C–O distances. This is in general agreement with spectroscopic and *X*-ray evidence for the type of hydrogen bridge involved in these structures, which has a length (oxygen–oxygen distance) of about 2·7 A.

In trifluoroacetic acid the relatively high scattering power of the CF_3 group prevented a full determination of structure. The C–O distances could not be determined individually, but the average is estimated at 1·30 A. The C–F distance of 1·36 A. and the reduced C–C distance of 1·48 A. are in agreement with other observations on such bonds. It is rather surprising that the tendency towards dimerisation and also the length of the hydrogen bridge are apparently but little affected by the increased strength of this acid.

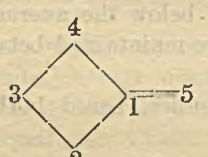
Karle and Brockway also examined deuterium acetate dimer but no difference could be observed between the qualitative appearance of the photographs obtained and those of acetic acid. Although an isotope effect (increase in length of the hydrogen bridge on substitution of deuterium for hydrogen) as great as that observed in oxalic acid dihydrate [7] might be detected by electron diffraction, yet it is unlikely that the acetic acid bridge would display such a large effect. The effect, if any, would more likely be

[7] J. M. Robertson and A. R. Ubbelohde, *Proc. Roy. Soc.*, 1939, *A*, **170**, 222.

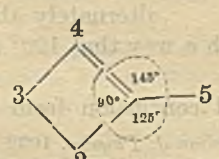
comparable to those observed in succinic and benzoic acids,[7] which are small. A hydrogen bridge of length 2·76 A. is not expected to give rise to a large isotope effect.

Organic Sulphonates.—A series of interesting single-crystal measurements have been made by L. M. Jensen and E. C. Lingafelter [8] on the quarter-hydrates of sodium 1-octane-, 1-decane-, 1-dodecane-, 1-tetradecane-, 1-hexadecane-, and 1-octadecane-sulphonate. In each case the space group is C_s^4 (Aa) or more probably C_{2h}^6 ($A2/a$), and the unit cell contains 32 molecules of the hydrate or 8 molecules of $4R{\cdot}SO_3Na,H_2O$. The cross-sectional area of the molecules is somewhat greater than that of sodium stearate or stearic acid, and decreases uniformly from 20·99 A.^{2} in the octane derivative to 20·08 A.^{2} for the octadecane derivative; at the same time the β angle increases by about 6°. The *c*-axis increases in steps of 10·2 A. from 55·39 to 106·46 A., which corresponds to an increase of eight carbon atoms along the *c*-axis for every additional two carbon atoms in the chain. Typical dimensions are: $a = 16{\cdot}86$, $b = 10{\cdot}17$, $c = 55{\cdot}39$ A., $\beta = 101°\,39'$ for $C_8H_{17}{\cdot}SO_3Na,\frac{1}{4}H_2O$, and $a = 16{\cdot}73$, $b = 10{\cdot}05$, $c = 106{\cdot}46$ A., $\beta = 107°\,13'$ for $C_{18}H_{37}{\cdot}SO_3Na,\frac{1}{4}H_2O$. Such a good series of single crystals of long-chain compounds has seldom been available for *X*-ray analysis.

Four-membered Rings.—Methylene*cyclo*butane (I) and 1-methyl*cyclo*butene (II) have been studied by W. Shand, V. Schomaker, and J. R. Fischer,[9] by the gas-diffraction method. These two structures are so similar that a unique determination by means of electron diffraction alone would be difficult or impossible. The diffraction evidence, however, is sufficient to establish quite definitely that neither of the compounds can have the *spiro*-pentane structure which has recently been advocated by F. Rogowski.[10] This is in agreement with the chemical evidence. The final results of the present investigation indicate coplanar ring structures. For (I) the distances are C–C = 1·55 ± 0·02 A., C=C = 1·34 ± 0·03 A., and angle $C_4C_1C_2 = 92{\cdot}5°$ ± 2°, in very good agreement with values obtained earlier by S. H. Bauer and J. Y. Beach.[11] For (II) C–C = 1·54 ± 0·03 A., C=C = 1·34 ± 0·03 A., angle $C_5C_1C_2 = 125° \pm 4°$, angle $C_4C_1C_2 = 93°\ 40' \pm 3°$. Symmetrical ring structures ± 3° distortion are thus indicated in both cases. In (II) the bond distortion appears to be distributed equally between the external C–C–C and C=C–C angles.



(I.)



(II.)

Polyphenyls and Phenylenes.—The type of binding between aromatic groups is a matter of considerable interest, but the evidence available has

[8] *J. Amer. Chem. Soc.*, 1944, **66**, 1946. [9] *Ibid.*, p. 636. [10] *Ber.*, 1939, **72**, 2021.
[11] *J. Amer. Chem. Soc.*, 1942, **64**, 1142; see *Ann. Reports*, 1942, **39**, 104.

hitherto been rather unsatisfactory. The crystal structures of diphenyl,[12] *p*-diphenylbenzene,[13] *o*-diphenylbenzene,[14] 1 : 3 : 5-triphenylbenzene,[15] and 4 : 4′-diphenyldiphenyl [16] have all been examined, but not with the accuracy available in modern technique, and individual bond lengths remain uncertain. In these early studies the ring size is generally given as 1·41 or 1·42 A., and the connecting bond lengths as about 1·48 A. In diphenylene more recent electron-diffraction results [17] give a ring size of 1·41 ± 0·02 A., and connecting links of 1·46 ± 0·05 A.

(Miss) I. L. Karle and L. O. Brockway [18] have now made a careful study of diphenyl, *o*-diphenylbenzene, and tetraphenylene by the electron-diffraction method. Such structures are, of course, much too complex to yield anything like unique solutions by the gas-diffraction method. The process rather consists of showing that a certain model, or sometimes several different models, are compatible with the data, while other models are not. But the method is very sensitive, and changes of bond length amounting to 0·02 or 0·03 A. are frequently sufficient to destroy the agreement for a given model. In diphenyl three different models satisfy qualitatively the appearance of the photographs. The first is planar, with ring size 1·39 A. and inter-ring distance 1·54 A. The second has a slightly distorted benzene ring and an inter-ring distance of 1·48 A., while the third is non-planar with free rotation of one ring with respect to the other. It is concluded that a non-planar structure for diphenyl is the most probable, especially as it avoids steric hindrance between the *o*-hydrogen atoms. The most probable average ring distances are 1·39 ± 0·02 A., and inter-ring distance 1·52 ± 0·04 A.

In *o*-diphenylbenzene the molecule cannot possibly be planar, and the average position of the two attached rings is thought to be orthogonal to the central ring, with possible oscillation of 15° from the normal position. The same bond distances apply.

(III.)

In tetraphenylene (III) all planar models proved unsatisfactory. A good solution was obtained from a model with four regular (1·39 A.) benzene rings and a non-planar *cyclo*octatetraene ring. In the latter the bonds alternate in length around the ring, between 1·39 A. and 1·52 A., the former of course being the sides of the benzene rings. The benzene rings are directed alternately above and below the average plane of the molecule in such a way that 120° angles are maintained between every pair of bonds.

The general conclusion from these studies, based both on inter-ring

[12] J. Dhar, *Indian J. Physics*, 1932, **7**, 43.

[13] (Miss) L. W. Pickett, *Proc. Roy. Soc.*, 1933, *A*, **142**, 333.

[14] C. J. B. Clews and (Mrs.) K. Lonsdale, *ibid.*, 1937, *A*, **161**, 493.

[15] B. P. Orelkin and (Mrs.) K. Lonsdale, *ibid.*, 1934, *A*, **144**, 630; (Mrs.) K. Lonsdale, *Z. Krist.*, 1937, **97**, 91.

[16] (Miss) L. W. Pickett, *J. Amer. Chem. Soc.*, 1936, **58**, 2299.

[17] See *Ann. Reports*, 1943, **40**, 92.

[18] *J. Amer. Chem. Soc.*, 1944, **66**, 1974.

distances and on the non-coplanarity of the molecules, is that the conjugation effects between adjacent aromatic rings are less than had hitherto been thought, and considerably less than between double or treble bonds and aromatic rings.[19] More definite conclusions must await really detailed *X*-ray investigations of the crystal structures.

A preliminary *X*-ray examination of crystals of triphenylmethyl chloride and bromide has been made.[20] They belong to the hexagonal system, and the space group is either C_{3i}^1–$H\bar{3}$ or C_3^1–$H3$, with six molecules in the hexagonal unit cell. The structures are obviously fairly complex, and although the halogen atoms have been located approximately by evaluating the Patterson functions, yet nothing definite can so far be said about the configuration of the triphenylmethyl group in these crystals.

Coronene.—A preliminary *X*-ray analysis of the crystal structure of the aromatic hydrocarbon coronene (IV) has succeeded in determining the position of the carbon atoms with considerable certainty by means of a two-dimensional Fourier projection of the structure along the monoclinic *b* crystal axis.[21] At the present stage of the analysis the interatomic distances cannot be measured very accurately, but, a strictly planar structure being assumed, there is already evidence that the average C–C distance is slightly greater than the accepted value for benzene (1·39 A.), and may be rather nearer to the graphite value of 1·42 A. The arrangement of the molecules in the crystal is such that it should ultimately be possible to make very accurate measurements of all the C–C distances.

(IV.)

These findings are of considerable interest in view of detailed calculations recently carried out by C. A. Coulson by the method of molecular orbitals on the coronene bond orders and lengths.[22] He has computed the energies of the mobile electrons in terms of the fundamental resonance integral β,[23] and from these results it is possible to obtain the bond orders and lengths relative to the standard values for C–C, C=C, and C≡C in ethane, ethylene, and acetylene. The calculated bond lengths are as follows :

	Coronene.	Graphite.	Benzene.
Mean length of C–C bonds (A.)	1·406	1·417	1·389
Length of central bonds (A.)	1·418	1·417	1·389

For structures of high symmetry and links between atoms of the same kind such calculations are obviously capable of considerable refinement. At present the experimental measurements are not nearly good enough to provide an adequate test of the theory, but we may perhaps hope for some improvement in this direction.

[19] J. M. Robertson and (Miss) I. Woodward, *Proc. Roy. Soc.*, 1937, *A*, **162**, 568; 1938, *A*, **164**, 436.

[20] S. N. Wang and C. S. Lu, *J. Amer. Chem. Soc.*, 1944, **66**, 1113.

[21] J. M. Robertson and J. G. White, *Nature*, 1944, **154**, 605.

[22] *Ibid.*, p. 797.

[23] *Proc. Roy. Soc.*, 1939, *A*, **169**, 413.

Other Structures.—Other investigations in the field of organic structures which may be briefly mentioned are unit cell and space-group determinations for phloroglucinol dihydrate,[24] the low-temperature form of abietic acid,[25] codeine and β-methylmorphimethine.[26] Ferritin and *apo*ferritin [27] are both face-centred cubic and contain the same protein. Although the cell size is the same, the *X*-ray intensities are different. The iron atoms in ferritin apparently occupy interstices between the protein molecules.

The structure of copper *NN*-di-*n*-propyldithiocarbamate has been studied, and the copper shown to have planar co-ordination.[28] Phytomonic acid, $C_{20}H_{40}O_2$,[29] has been shown to have a relatively long chain structure and a single side chain, probably a methyl group. J. M. R.

v. *Natural and Synthetic Fibre Structures.*

Introduction.—Fibre structures have not recently been reviewed as such in these Reports. The present note covers especially the last two years, mainly from the *X*-ray standpoint. Metals, complex biological fibres, and applied aspects are omitted. The unity of macromolecular studies has been reflected in the co-ordination of all relevant physico-chemical techniques in their problems, which are as basic to colloid science, medicine, and biology as to the industries of textiles, soaps, plastics, and rubbers. Dominant features are the increasing tendency to treat long-chain molecules from fundamental first principles; the extension to fibres of ideas and results (energetics and structure) from the simpler metal and *n*-aliphatic chain systems; the growing emphasis on secondary structure, aided by new techniques such as electron microscopy and small-angle *X*-ray scattering; and the success in interpreting physical and especially mechanical fibre properties on a molecular basis. Surveys, varying conditions and environment, still yield more fundamental results with fibres than separate precision structure analyses.

New Sources.—High-polymer studies have brought about the formation of sub-groups of physico-chemical societies,[1] increasingly participate in others, and have reoriented a vast applied literature. New governmental and research associations [2] and two complete abstracting digests [3] deal exclusively with fibres, whose structural problems have been focused in four

[24] C. R. Bose and R. Sen, *Indian J. Physics*, 1943, **17**, 163.

[25] H. S. Shdanov, M. J. Lazarev, and N. G. Sevastianov, *Compt. rend. Acad. Sci. U.R.S.S.*, 1941, **31**, 767.

[26] L. Castelliz and F. Halla, *Z. Krist.*, 1943, **105**, 156.

[27] I. Fankuchen, *J. Biol. Chem.*, 1943, **150**, 57.

[28] G. Peyronel, *Gazzetta*, 1943, **73**, 89.

[29] S. F. Velic, *J. Biol. Chem.*, 1944, **165**, 101.

[1] *E.g.*, Division of High Polymer Physics, Amer. Physical Soc., June, 1944; British Rheologists' Club, 1940.

[2] *E.g.*, Southern Agric. Res. Stn., U.S.A.

[3] "Natural and Synthetic Fibres," M. Harris and H. Mark, Intersci. Publ. Inc., 1944; "Resins, Rubbers, and Plastics," H. Mark and E. S. Proskauer, Intersci. Publ. Inc., 1942.

recent symposia.[4] Annual reviews have been found necessary in the protein field alone.[5] Among new journals should be noted *J. makromol. Chem.*; of books and surveys, Vols. 4, 5 of the "High Polymer" series are fibre-structural landmarks among many.[6]

The Fibrous State.—Fibres are imperfectly ordered solids, occurring usually as discrete filaments, though also in compact form. Genesis and growth are discussed by W. Ostwald.[7] They are formed of long-chain molecules, usually high polymers, but comprise also sheet-like plates linked in columnar form,[8] by stress-interleaving,[9] or by mere van der Waals forces.[10] Fibres of animal and plant origin have a histological and cytological structure : in wool the fibre structure and properties are those of the cortical cell.[11] Chemical composition may vary longitudinally in a single fibre, and markedly so as between medulla, cortex, and scale.[12] Various degrees of order are obtainable with chains whose packing is a function of shape, mobility, and local intermolecular forces. The fringed-micelle theory [13] is now preferred to the crystallite [14] and continuous structure [15] pictures. In this, a single chain may thread its way through alternate crystalline and amorphous regions which really differ only in degree, and alter (though with more than cybotactic permanence) with temperature, stress, pressure, swelling, etc. The crystalline nuclei give a brittle strength, the amorphous tangle tenacity and resilience of orientation, with some plastic flow. Fibre structure analysis has therefore to deal not only with the phase-ordered crystallites, but also with their shape, size, and relation to the matrix. Special orient-

[4] "Mol. Wt. Distrn. in High Polymers," *Trans. Faraday Soc.*, 1944, **40**, 217; "Fibre Structures," *X*-Ray Anal. Gp., Inst. of Phys., Mtg. Oxford (1944); "Physics of Rubber and other H.P.'s," Amer. Physical Soc., cf. *J. Appl. Physics*, 1944, **15**; "Physical Chemistry of Proteins," *Chem. Rev.*, 1942.

[5] Cf. Annual "Advances in Protein Chemistry," M. L. Anson and J. T. Edsall, 1944, I, Acad. Press Inc., N.Y.; "Advances in Colloid Science," 1942, I; "Advances in Enzymology," I, II, Intersci. Publ. Inc., N.Y.; "Review of Biochemistry," Stanford Univ. P.O., California.

[6] "Natural and Synthetic H.P.'s," 1942, 708 pp., K. H. Meyer, Intersci. Publ.; "Cellulose and Cellulose Derivatives," 1943, 1196 pp., E. Ott, Intersci. Publ.; cf. also "Physical Chemistry of H.P. Systems," 1940, 353 pp., H. Mark, Intersci. Publ.; "Elastic and Creep Properties of Filamentous Materials and other H.P.'s," 1943, H. Leaderman, Text. Fdn. Washington;] *Rep. Prog. Physics*, L. R. G. Treloar, 1942—43, **9**, 113; G. Gee, *Ann. Reports*, 1942, **39**, 7; J. Needham *et al.*, *J. Gen. Physiol.*, 1944, **27**, 201.

[7] *Kolloid-Z.*, 1943, **102**, 35.

[8] *E.g.*, Sodium thymonucleate, W. T. Astbury, *Sci. Progr.*, 1939, **133**, 1.

[9] *E.g.*, Chrysotile asbestos, E. Aruja, *J. Sci. Instr.*, 1944, **21**, 115.

[10] *E.g.*, Fibrous carbon, J. Gibson, H. L. Riley, and J. Taylor, *Nature*, 1944, **154**, 544.

[11] H. J. Woods, *Proc. Roy. Soc.*, 1938, *A*, **166**, 76.

[12] J. B. Speakman, *J. Text. Inst.*, 1941, **32**, 3.

[13] O. Gerngross, K. Herrmann, and W. Abitz, *Biochem. Z.*, 1930, **228**, 409; K. Herrmann and O. Gerngross, *Kautschuk*, 1932, **8**, 181.

[14] K. H. Meyer and H. Mark, "Der Aufbau der H.P. in org. Naturst.," 1930; H. Mark, *J. Physical Chem.*, 1940, **44**, 764.

[15] H. Staudinger, "Die hochmol. org. Verbind. Kautschuk u. Cellulose," 1932.

ations may occur in cell-wall structures;[16] normally, the crystallites are long rods or ribbons with random radial orientation, but approximately parallel to the fibre axis; biaxial orientation may be securable in rolled film.[17] *X*-Radiograms taken perpendicular to the fibre axis are thus intermediate to rotating-crystal and powder *X*-radiograms, the arcing of reflections depending on the degree of tolerance between crystallite and fibre axes. For this reason, meridian reflections, barred in crystal rotation photographs, often appear. Relative intensities need special settings or calculation.

Technique.—Fibre cameras are best fitted with stretching frames and conditioning gadgets;[18] screening foil and vacuum chamber minimise general scattering from air and amorphous fibre regions; monochromatised radiation, as in the arrangements of A. Guinier[19] or I. Fankuchen,[20] is vital for small-angle scattering and ordinarily preferable. Moving cameras such as those of O. Kratky[21] simplify interpretations, the geometry of which has been summarised by Y. Go.[22] Of recent photometers, that of N. H. Chamberlain[23] has many fibre applications. For speedy intensity recording of uniaxial fibre spectra, the G-M counter[24] has advantages. High resolution being required for the fine detail of macromolecular structure and texture, fine slits permitting registration up to >600 A. are now frequent practice,[25] especially in conjunction with high-power *X*-ray generators.[26] In this connection, precision beam focusing,[27] permitting the origin of *X*-rays to function as the initial slit, enhances useful intensity. *X*-Ray power is expedient in allowing quick comparative study of homologues,[28] or of fibres maintained at successive small intervals of humidity, pH, swelling, temperature, pressure, extension, etc.; it extends the scope of *X*-ray methods in following such topochemical reactions as mercerisation[29] or nitration of cellulose.[30] The use of glass-capillary slits (0·03 mm. in diameter) providing mirror reflection has been extended to the micro-analysis of single fibres and the drawing process.[31] Of special fibre interest is the application of diffuse low-angle scattering to problems of grain size and texture (*q.v.*), and of the analytical technique for statistical arrangements such as mixed crystallisation, intercalation, or turbostratic layering in swelling studies. The local chemical

[16] R. D. Preston, *Biol. Rev.*, 1939, **14**, 281.
[17] C. W. Bunn, *Trans. Faraday Soc.*, 1939, **35**, 482.
[18] W. T. Astbury, unpublished.
[19] *Compt. rend.*, 1937, **204**, 1115; *Ann. Physique*, 1939, **12**, 161.
[20] *Nature*, 1937, **139**, 193.
[21] O. Kratky, F. Schossberger, and A. Sekora, *Z. Elektrochem.*, 1942, **48**, 409.
[22] *Bull. Chem. Soc. Japan*, 1940, **15**, 239. [23] *J. Text. Inst.*, 1944, **35**, T61.
[24] A. Eisenstein and N. S. Gingrich, *Rev. Sci. Instr.*, 1941, **12**, 582.
[25] *E.g.*, R. Hosemann, *Z. Elektrochem.*, 1940, **46**, 535.
[26] I. MacArthur, *Electron. Eng.*, 1944, **17**, 272; 1945, **17**, 317; W. T. Astbury and I. MacArthur, *Nature*, 1945, **155**, 108.
[27] A. Guinier and J. Devaux, *Rev. Sci.*, 1943, 341; *Compt. rend.*, 1943, **217**, 682.
[28] I. MacArthur, Mtg. *X*-Ray Anal. Gp., Inst. of Phys., Oxford (1944).
[29] E. Green, Ph.D. Thesis, Leeds University (1938).
[30] M. Mathieu, *Compt. rend.*, 1941, **212**, 80.
[31] I. Fankuchen and H. Mark, *J. Appl. Physics*, 1944, **15**, 364.

composition and density of reflecting regions, required in structure analyses, are not necessarily easy determinations. Valuable analytical developments are new and improved methods for amino-acids [32] (protein fibres); the electron probe with its transmitted velocity spectrum peaked by the excitation of *X*-ray K, L, or M levels [33] will be of future interest. Porosity, preferential swelling, and adsorption of the immersion medium by the non-crystalline matrix are germane in density determinations; comparative results of the usual immersion methods and the helium procedure of P. M. Heertzes [34] are described.[35]

Inorganic.—Common alumina and silicate fibres are of membrane type with liquid content.[36] Technical methods described for producing (*e.g.*, in glass) suitable strength and texture include high temperature centrifugal spinnerets, and surface and admixture treatments.[37] Filamentous carbon, prepared by cracking of methane, has given a fibre *X*-radiogram,[10] the lamellar hexagon layers, ~ parallel to the fibre axis, showing the usual 3·5 A. graphitic spacing. A pioneer in the technique of diffuse scattering, A. Guinier extends the work on amorphous carbons and coal,[38] determining micelle dimensions and distribution; as expected, activated carbons show a very fine state of division which varies with treatment.[39] Similar studies on fibrils of chrysotile [40] reveal the hexagonal close-packing of parallel rods, earlier found in *n*-paraffins [41] and *n*-alcohols,[42] in a discrete pair of long spacings of ratio $\sqrt{3}$: 1. Fibril diameters vary between 195 and 250 A. Further independent work on chrysotile asbestos,[9, 43] reveals novel features. The monoclinic cell has *a* 14·64, *b* 9·22, *c* (fibre axis) 5·33 A., β 93·2°. The Si_4O_{11} chains originally proposed yield place to a duplex sheet structure of $Si_2O_5Mg_3(OH)_4$ consisting of a branch-type sheet of $O_4(OH)_2Mg_6(OH)_6$ linked to $O_6Si_4O_4(OH)_2$ (hexagonal net of tetrahedral SiO_4 with OH in the plane of the tops of the tetrahedra) by the common $O_4(OH)_2$. Certain diffuse reflections reveal irregularly stacked packing: their positions and asymmetric nature accord with two-dimensional net scattering [44] and, together with those of the congener antigorite, have been used by E. Aruja to interpret fine detail. Cell and space-group of gümbelite [45] are determined. An

[32] A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, 1941, **35**, 91, 1358; A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *ibid.*, 1944, **38**, 65; R. Consden, A. H. Gordon, and A. J. P. Martin, *ibid.*, p. 224; J. R. McMahon and E. E. Snell, *J. Biol. Chem.*, 1944, **152**, 83; A. C. Chibnall *et al.*, *Biochem. J.*, 1943, **37**, 354, 360, 372.

[33] J. Hillier and R. F. Baker, *J. Appl. Physics*, 1944, **15**, 663.

[34] *Rec. Trav. chim.*, 1933, **52**, 305.

[35] *Idem, ibid.*, 1941, **60**, 91, 329; 1942, **61**, 751; R. P. Rossman and W. R. Smith, *Ind. Eng. Chem.*, 1943, **35**, 972.

[36] W. Ostwald, *Kolloid. Z.*, 1943, **102**, 181.

[37] B.P. 563,678; U.S.P. 2,338,473, 2,331,944/5/6.

[38] B.C.U.R.A. Conference, 1943, London; *Proc.*, 1944.

[39] H. Brusset, J. Devaux, and A. Guinier, *Compt. rend.*, 1943, **216**, 152.

[40] I. Fankuchen and M. Schneider, *J. Amer. Chem. Soc.*, 1944, **66**, 500.

[41] A. Müller, *Proc. Roy. Soc.*, 1932, *A*, **138**, 514.

[42] I. MacArthur, unpublished. [43] B. E. Warren, *Amer. Min.*, 1942, **27**, 235.

[44] *Idem, Physical Rev.*, 1941, **59**, 693. [45] E. Aruja, *Min. Mag.*, 1944, **27**, 11.

interesting discussion of gelatinising silicates in terms of lateral radical and network structure is made by K. J. Murata.[46]

n-*Aliphatic Unbranched Chains.*—(a) *Soaps.* Soaps fibre with the paraffin chains perpendicular, not parallel, to the fibre axis.[47] Further *X*-ray evidence [48] confirms their existence in gel or solution as head-to-head bimolecules of standard chain cross-section, giving "long spacings" (L) which increase on dilution, or on addition of benzene or dye solubilisation, by intercalation at heads and tails respectively. Micelle aggregates are lamellæ,[49] with the dominant cohesion along the smallest axis. As with montmorillonite, thixotropy is evidenced, *e.g.*, by magnesium stearate in benzene,[50] the temperature of the sol–gel transformation increasing linearly with soap concentration. The equilibrium has been studied spectrophotometrically.[51] The most direct evidence of gel structure and fibre size and texture is still the excellent electron micrographs (EM) for sodium laurate,[52] statistical analysis of whose interlocked mesh of ribbon fibrils showed a multi-pile lamellar structure of parallel, slightly hydrated, bimolecular chains. Fibering of metal soaps in mineral oil [53] is favoured by free acid. Calcium and aluminium cations yield very small fibres, but, for sodium soaps, large fibres are favoured by low-viscosity polar oils, unsaturated soap chains, and shearing stress in combination with polar additants such as glycerol. Growth mechanisms and energetics have been discussed.[53, 54] Transparent soap is not amorphous, but is composed of ultra-fine random fibres (γ-form, *q.v.*) with free glycerol.[55]

Characterisation of the numerous phase structures and their stability ranges and transitions, complicated as they are by water content, continues by *X*-ray and thermal methods. For the sodium palmitate–water system F. G. Chesley [56] finds six transitions. In addition to the liquid-crystalline state, four crystalline modifications have been given *X*-ray criteria for the sodium stearate–water system [57] and many well-known features of long aliphatic chains with polar end-groups have been confirmed and extended by fibre *X*-radiograms.[58] Transitions variously indicate change of water content,

[46] *Amer. Min.*, 1943, **28**, 545.

[47] S. Ross, *J. Physical Chem.*, 1942, **46**, 414; O. E. A. Bolduan, J. W. McBain, and S. Ross, *J. Physical Chem.*, 1943, **47**, 528.

[48] H. Kiessig, *Kolloid-Z.*, 1941, **96**, 252; J. W. McBain and K. E. Johnson, *J. Amer. Chem. Soc.*, 1944, **66**, 9.

[49] W. Philippoff, *Kolloid. Z.*, 1941, **96**, 255.

[50] B. S. Kandelaki, *Izv. Gruz. Ind. Inst. Kirova*, 1940, **13**, 109.

[51] J. Stauff, *Z. Elektrochem.*, 1941, **47**, 820.

[52] L. Marton, J. W. McBain, and R. D. Vold, *J. Amer. Chem. Soc.*, 1941, **63**, 1990.

[53] W. Gallay and I. E. Puddington, *Canadian J. Res.*, 1944, *B*, **22**, 66, 90, 103.

[54] J. Stauff, *Kolloid-Z.*, 1941, **96**, 244.

[55] J. W. McBain and S. Ross, *Oil and Soap*, 1944, **21**, 97.

[56] *J. Chem. Physics*, 1940, **8**, 642.

[57] R. H. Ferguson, F. B. Rosevear, and R. C. Stillman, *Ind. Eng. Chem.*, 1943, **35**, 1005.

[58] J. W. McBain, O. E. A. Bolduan, and S. Ross, *J. Amer. Chem. Soc.*, 1943, **65**, 1873; J. W. McBain, A. de Bretteville, and S. Ross, *J. Chem. Physics*, 1943, **11**, 179.

change of inclination of paraffin chain axes to polar layer planes, genotypic transformations (1-dimensional melting), confirmed by the sudden variation in thermal expansion and onset of effects on the surface tension and viscosity of surrounding polar oils,[59] and the inception at the waxy-superwaxy transition of the hexagonal symmetry of " molecular rotation." [60] Temperature-spectra showing the latter effect together with some L variation have been made for sodium stearate.[61] R. H. Ferguson believes the water is present only in solid solution ; [57] M. J. Buerger,[62] using de Jong and precision Weissenberg technique on sodium stearate crystals prepared by A. de Bretteville, has determined the α, β, γ forms as $-\frac{1}{2}H_2O$, $-\frac{1}{8}H_2O$, and anhydrous respectively. Sodium stearate, $\frac{1}{2}H_2O$ is monoclinic, a 9·16, b 8·00, c 103·96 A., β 93° 43′, space-group A2/a. The variety of crystal forms and packing detail adopted by n-aliphatic polar molecules is much wider than is commonly supposed. While broad essentials are obtainable from fibre and powder X-radiograms, particularly by use of the anisotropic thermal expansion in temperature-spectra,[28] complete crystallographic analyses, such as those of A. Müller [63] and C. W. Bunn,[17] could well be extended to other forms in a field where so much understanding of molecular structure and forces can be gained by the precise use of X-ray, thermal, polarisation, and force-field methods.

(b) n-*Paraffins*. While higher paraffins fibre with some tenacity, lower members give moderately tough and rubbery fibres only on admixture, *e.g.*, with lithium stearate.[64] Work continues on the lines of A. Müller and A. R. Ubbelohde, crystalline forms, transitions, stability ranges, and pre-melting being discussed by C. G. Gray, A. van Hook, and H. Fröhlich.[65] Dielectric measurements extend previous experimental results; molecular flexure, invoked to account for the entropy changes involved, is found such as to leave ~ half the chains untwisted at the second-order transition. The recent infra-red conclusion that in polythene some branching occurs—in particular ~2% of methyl groups [66]—may bear on certain systematic anomalies met [17] in its crystal structure determination.

Branched-chain Hydrocarbons and Rubbers.—(a) *General and Macro-structure*. Rubber-like materials consist of long flexible chains weakly cross-linked. They are distinguished from ordinary liquids and solids respectively by these few links (chemical, vulcanised, sterically knotted) and by their weak control of form through entropy rather than energy. Local crystallisation, impeded by plasticisers and temporary knots, is promoted by

[59] W. Gallay and I. E. Puddington, *Canadian J. Res.*, 1943, *B*, **21**, 211, 225.

[60] L. Pauling, *Physical Rev.*, 1930, **36**, 430; T. E. Stern, *Proc. Roy. Soc.*, 1931, *A*, **130**, 551.

[61] A. de Bretteville and J. W. McBain, *J. Chem. Physics*, 1943, **11**, 426.

[62] M. J. Buerger, A. de Bretteville, F. V. Ryer, and L. B. Smith, *Proc. Nat. Acad. Sci.*, 1942, **28**, 526; M. J. Buerger, *ibid.*, p. 529.

[63] *Proc. Roy. Soc.*, 1928, *A*, **120**, 437.

[64] Foote Min. Co., *Rev. Sci. Instr.*, 1944, **15**, 157.

[65] H. Fröhlich, *Trans. Faraday Soc.*, 1944, **40**, 498; A. van Hook and L. Silver, *J. Chem. Physics*, 1942, **10**, 686; C. G. Gray, *J. Inst. Petr.*, 1943, **29**, 226.

[66] H. W. Thompson, *J.*, 1944, 183.

addition of active fillers such as carbon or dry silica.[67] Normally, crystallites are absent or randomly oriented; stretching favours fibering.

Secondary structure in such systems, in particular its relation to elasticity, has been widely studied, mainly by statistical and thermodynamic methods.[68] In such treatments, the properties of bulk polymer are usually referred to those of individual Gaussian or free-link chains involving the chain length (as determined by osmotic pressure, viscosity, or light scattering [69] methods) or its mean end-to-end distance (A). A is unaffected by a symmetric hindrance potential; [70] for unsymmetric steric effects, the form of the A distribution function remains unchanged, but its magnitude increases.[70] C. Sadron's treatment [71] replaces the free-link chain by its "equivalent particle," a time-average of its mean geometric articulation; this surface of revolution, whose shape reflects chain rigidity, is then treated by Brownian movement methods. Fundamental constants of diffusion, orientation, translation, viscosity, and temperature effects are expressible. Actual cases require individual correction because of the special importance of steric and polar factors.[72] Model experiments on threaded beads to simulate the effect of varying chain length, concentration, solvent nature, etc.,[73] give general confirmation.

An interesting treatment of the melting of high polymers [74] explains in terms of degree of polymerisation (P), latent heat per link, crystalline-amorphous ratio, and structural co-ordination numbers—(*a*) convergence of melting points with increase in P, (*b*) melting ranges, (*c*) stable coexistence of amorphous and crystalline regions in a composite phase; data by N. Bekkedahl [75] corroborate for rubber. Recent determinations of crystallite size (rubber) have been made by means of line-broadening (A. Taylor's analysis [76]) and small-angle diffuse scattering.[31, 77] The values (80, 52—81 A. perpendicular to, ~300 A. parallel to, stretch) confirm older figures.[78] EM determinations show a gel fibril structure of minimum width 100—400 A., but, in

[67] P. Rebinder, G. A. Ab, and S. J. Veiler, *Comp. rend. Acad. Sci., U.R.S.S.*, 1941, **31**, 444; C. E. Hall *et al.*, *Ind. Eng. Chem.*, 1944, **36**, 634.

[68] H. M. James and E. Guth, *J. Chem. Physics*, 1943, **11**, 455, 531; F. H. Müller, *Angew. Chem.*, 1940, **53**, 425; *Kolloid. Z.*, 1941, **96**, 326; W. and H. Kuhn, *Helv. Chim. Acta*, 1943, **26**, 1394; F. T. Wall, *J. Chem. Physics*, 1943, **11**, 527; P. J. Flory and J. Rehner, *J. Chem. Physics*, 1943, **11**, 512; R. Simha, *Ann. N.Y. Acad. Sci.*, 1943, **44**, 297; J. J. Hermans, *Kolloid. Z.*, 1943, **103**, 210; J. D. Ferry, *Ann. N.Y. Acad. Sci.*, 1943, **44**, 313; J. J. Press and H. Mark, *Rayon Text. Monthly*, June—Aug., 1943; R. Houwink, *J. Physical Chem.*, 1943, **47**, 436; L. R. G. Treloar, *Trans. Faraday Soc.*, 1944, **40**, 109; and especially ref. 6.

[69] P. Debye, *J. Appl. Physics*, 1944, **15**, 338; W. W. Lepeschkin, *Kolloid. Z.*, 1943, **105**, 141.

[70] P. J. Flory and J. Rehner, *Ann. N.Y. Acad. Sci.*, 1943, **44**, 419.

[71] C. Sadron, *J. Phys. Radium*, 1943, **14**, 92.

[72] C. W. Bunn, *Proc. Roy. Soc.*, 1942, *A*, **180**, 67, 82.

[73] H. A. Stuart, *Naturwiss.*, 1943, **31**, 123.

[74] E. M. Frith and R. F. Tuckett, *Trans. Faraday Soc.*, 1944, **40**, 251.

[75] *J. Res. Nat. Bur. Stand.*, 1935, **15**, 503. [76] *Phil. Mag.*, 1941, **31**, 339.

[77] S. D. Gehman and J. E. Field, *J. Appl. Physics*, 1944, **15**, 371.

[78] J. Hengstenberg and H. Mark, *Z. Krist.*, 1928/9, **69**, 271.

addition, amorphous nodules also appear, often of considerable size.[79] For a standard 600% stretch in rubber, crystalline fraction and tensile strength increase as crystallite size decreases. The increase in crystallisation of rubber with stretch is determinable by the parallel increases of magnetic anisotropy [80] and fibre *X*-radiogram intensity. Lattice size is very constant during stretch and density increases $\gtrsim 1\%$.[81] Cell expansion with temperature is anisotropic.[78]

Important results on the phenomena of second-order transitions with their time effects and structural implications are those of R. F. Boyer and R. S. Spencer [82] (rubbers, nylon, polythene, etc.) and of H. Mark *et al.*[83] (polystyrene). The former authors, for polymers, copolymers, and mixtures, determine by dilatometry the rates of expansion (β_1, β_2) below and above the transition point (T_t); the relations found are applicable to the determination of crystalline-amorphous ratios, nature of phases, and something of the variation in P. Mark finds that above the polystyrene T_t, the specific volume V–T curve is fixed; below T_t, $\partial V/\partial T$ is fixed ($= \beta_1$) but V itself depends on the speed of cooling, T_t being higher the quicker the speed. On quick heating from the lower temperatures, any particular V–T curve is retraceable, but on maintaining T fixed in a wide range (40—80°) V falls to a minimum curve at a rate increasing with T. The phenomena, which recall comparable cyclical results of R. Buckingham [84] and, in another field, of W. A. Wood,[85] emphasise the effect of time of relaxation phenomena in long-chain molecules. This is vital in analysing load-extension and hysteresis phenomena, especially in textile fibres,[86] is instanced in mechanical depolymerisations by supersonic frequencies,[87] and also in T_t variations, which are closely related to viscosity.[88] T_t increases with increasing difficulty of movement. Thus T_t is raised by bulky groups or rings as side chains (polystyrene), by primary valency cross-linking (polydivinyl benzene), or by dipole forces (polyacrylic acid); T_t is lowered by mobile non-polar side chains (polybutadiene) or by screening of dipoles (polyacrylic esters). $T_t \times \beta_2 \cdot{=}\cdot$ const. for many series.[82]

(b) *Fine structure.* Structures for rubber, rubber hydrochloride, polychloroprene and β-guttapercha have been noted.[89] By electron diffraction of thin (250 A.) films of guttapercha, K. H. Storks finds the macromolecule in concertina-like folds.[90] Structures based on fibre *X*-radiograms with

[79] C. E. Hall, E. A. Hauser, D. S. LeBeau, F. O. Schmitt, and P. Talalag, *Ind. Eng. Chem.*, 1944, **36**, 634.
[80] E. Cotton-Feytis, *Compt. rend.*, 1942, **214**, 485; **215**, 299.
[81] W. H. Smith and N. P. Hanna, *J. Res. Nat. Bur. Stand.*, 1941, **27**, 229.
[82] *J. Appl. Physics*, 1944, **15**, 398.
[83] T. Alfrey, G. Goldfinger, and H. Mark, *ibid.*, 1943, **14**, 700.
[84] *Trans. Faraday Soc.*, 1934, **30**, 375.
[85] S. L. Smith and W. A. Wood, *Proc. Roy. Soc.*, 1941, *A*, **178**, 93.
[86] *E.g.*, H. Leaderman, ref. 6.
[87] G. Schmid and E. Beuttenmüller, *Z. Elektrochem.*, 1943, **49**, 325.
[88] E. Jenckel, *Kolloid. Z.*, 1942, **100**, 163.
[89] *Ann. Reports*, 1942, 108; 1943, 96. [90] *Bell Lab. Rec.*, 1943, **21**, 390.

limited and partly unresolved reflections cannot attain the precision of crystal analyses: G. A. Jeffrey's β-guttapercha structure [91] differs from that of C. W. Bunn in that a planar distribution of the C–C bonds about the double bond is found, with no distortion of the methyl group out of the plane; and orientation of the CH_2–CH_2 bond to the plane of the double bond is rather larger (80° *v.* 63°). The C-atom parameters listed yield the more normal bond lengths and angles of C–C 1·54, C=C 1·33 A., ∠C–C=C 122°, ∠C–C–C 109°.

Stretched poly*iso*butylene yields possibly the finest fibre *X*-radiogram yet found for high polymers. C. S. Fuller *et al.*[92] find a rhombic cell with *a* 6·94, *b* 11·95, *c* 18·03 A. From intensity analysis, two chains penetrate the cell, and the *iso*butyl residues are held to coil spirally, each CMe_2 group rotating 45° about the fibre axis (*c*) with respect to its predecessor to remove methyl group steric interference. It may be noted [93] that certain (00*l*) absences basic to this structure appear on permitting meridian reflection, that mutual methyl group interference is still considerable, and that some adjustment of the methyl groups brings the structure in closer agreement, on force-field theory, with the remarkable anomalies found for the heat of formation.[94]

Poly-ethers, -esters, -amides.—In these polymers the chain itself is modified by the insertion of oxygen or nitrogen atoms. The mutual adjustment between alkyl chain and polar layer forces depends on average P, and type, mobility, size, shape, density and distribution of the groups inserted. The force fields being differently responsive to variations in physical conditions, all grades of elasticity and plasticity occur, crystalline forms and transitions are numerous and individualistic, and amorphous, fibrous, mesomorphous, and crystalline regions occur.

When polar groups are small and their distribution dense and regular, good fibre diagrams are obtainable. Thus polyoxymethylenes $(-CH_2-O-)_n$ are hexagonal, C_3^2, with a fibre period of 17·25 A. Chain orientation is non-planar tub form with helical progression along the fibre axis.[95] Similarly, polyethylene oxide $(-CH_2-CH_2-O-)_n$ (I) is monoclinic, *a* 9·5, *b* (fibre axis) 19·5, *c* 12·0 A., β 101°; 4 chains per cell, 9 units per fibre period, involving again a folded chain.[95, 96] Less order is shown in *X*-ray and electron diffraction studies on methacrylate fibres.[97] With a low density of dipoles, the paraffin chain packing becomes more dominant; for the decamethylene series [98] (II) C. S. Fuller finds monoclinic types with normally-packed zigzag chains parallel and also inclined to the fibre axis. The cells of the tri-

[91] *Trans. Faraday Soc.*, 1944, **40**, 517.

[92] C. S. Fuller, C. J. Frosch, and N. R. Pape, *J. Amer. Chem. Soc.*, 1940, **62**, 1905; cf. also R. Brill and F. Halle, *Naturwiss.*, 1938, **26**, 12.

[93] W. T. Astbury and H. J. Woods, unpublished.

[94] M. Polanyi, unpublished.

[95] E. Sauter, *Z. physikal. Chem.*, 1933, *B*, **21**, 161, 186.

[96] H. and M. Staudinger, *ibid.*, 1937, *B*, **37**, 403.

[97] H. A. Robinson, R. Ruggy, and E. Slantz, *J. Appl. Physics*, 1944, **15**, 343; G. D. Coumoulos, *Proc. Roy. Soc.*, 1943, *A*, **182**, 166.

[98] *Chem. Rev.*, 1940, **26**, 143.

methylene poly-esters of dibasic acids usually consist of normally-packed paraffin chains lying parallel to the cell long axis (itself at ~30° to the fibre axis) with the planes of dipoles perpendicular to the fibre axis.[99] A slightly different end-group packing from those of (I), (II), is indicated by a lower L, but the same $=CH_2$ increment. Odd and even members show finer distinctions. On stretching the fibre, cells aline nearer the fibre axis, and equatorial and meridian reflections resolve to non-axial pairs. On strain release, the process is reversible. In the 1·16 member, chains show only the parallel alinement of the mesomorphic state characterised by the 4·2 A. lateral spacing.

Structural analyses have been made for several nylons,[100] especially the condensate of $NH_2{\cdot}[CH_2]_6{\cdot}NH_2$ and $CO_2H{\cdot}[CH_2]_4{\cdot}CO_2H$, but no satisfactory structure is yet released in detail. The strong equatorials at 4·4 and 3·8 A. conform with one type of paraffin chain packing; meridians indicate an axial periodicity (16·8 A.) rather less than that demanded by a vertical zigzag chain. R. Brill's unit cell [1] has a 9·66, b 8·32, c 17·2 A., γ 65°. Hydrogen-bonding at the CO·NH links emphasises the parallel with proteins. C. S. Fuller's extensive studies [2] attempt to relate structure and physical properties. The same features appear as in poly-esters, *e.g.*, the transition from extended to balanced retracted chains with inclination of chains to fibre axis a function of tension. Forms range over amorphous, mesomorphous, and fibrous, from coplanar dipoles with radially random chains to parallel chains with random dipoles. Co-polymerisation and weakening of polar forces (*e.g.*, by *N*-methylation) affect side-packing less than fibre-axial period. Increasing *N*-methylation lowers melting point and elastic modulus, increases water sorption, and broadens the 3·8 A. spacing. The retracted chain form is favoured by high temperature annealing, high *N*-methylation, low polar group density along the chain, relaxed longitudinal strain, swelling, and polar plasticisers. As with polythene, rolling (which induces the extended chain form) gives biaxial orientation. The mobility of such chains has been shown by many methods. For the condensate of adipic acid and $(CH_2)_6N_4$, R. Brill [3] finds the transition from monoclinic lattice to the hexagonal form characterising " rotating chains " to be lowered in temperature and speeded by moisture. Similar polarisation studies [4] to those on ketones [5] also confirm chain mobility, while spontaneous adjustments and coiling are seen in the electron diffraction of films,[90a] in film deposition [6] and in other phenomena.[2, 7]

Altering the balance of stresses in such systems may lead to macro-

[99] C. S. Fuller, *J. Amer. Chem. Soc.*, 1942, **64**, 154.

[100] I. Sakurada and I. Hizawa, *J. Soc. Chem. Ind., Japan*, 1940, **43**, *B*, 348; L. E. R. Taylor, unpublished.

[1] *Z. physikal. Chem.*, 1943, *B*, **53**, 61.

[2] W. O. Baker and C. S. Fuller, *J. Amer. Chem. Soc.*, 1942, **64**, 2399; 1943, **65** 1120.

[3] *J. pr. Chem.*, 1942, **161**, 49.

[4] W. O. Baker and W. A. Yager, *J. Amer. Chem. Soc.*, 1942, **64**, 2164, 2171.

[5] A. Müller, *Proc. Roy. Soc.*, 1937, *A*, **158**, 403; 1940, *A*, **174**, 137.

[6] F. H. Müller, *Kolloid. Z.*, 1943, **103**, 144.

[7] W. B. Bridgman and J. W. Williams, *J. Amer. Chem. Soc.*, 1937, **59**, 1579.

structures along the fibre axis sufficiently regular to give diffraction phenomena. For nylons and poly-esters respectively, macrospacings by H. Mark [31a] and by K. Hess and H. Kiessig,[8] which increase irreversibly on heating, and others by E. Ott [98a] for polyoxymethylenes have no relation to the well-defined fibre axis periods, but are of the order of crystallite size determined from electron diffraction.[9] Degraded nylon fibres do not yield the fine-diameter (50—100 A.) proto-fibrils so characteristic of natural celluloses.[10]

Carbohydrates.—Cellulose, with its great proportion of active polar groups and regular chain of cellobiose units, forms fibres with well-marked crystallites. P varies greatly, ~2000 being usual for untreated cotton, ramie, etc.,[11] compared with ~250, >1000, for starch amylose and amylopectin respectively.[12] Very long L are not found, though weak links at regular 2600 A. intervals have been claimed.[13] EM determinations of width [14] confirm the *X*-ray values (30—100 A.) for the finest fibrils. In EM's, the branched polysaccharide dextran shows periodic swellings at 800 A. intervals.[15] Three crystalline structures, cellulose, hydrate-cellulose (mercerised), and water-cellulose are well differentiated. The first native plant cellulose found in the mercerised state is the halicyst membrane.[16] Two types of cellulose cell are distinguished,[17] (a) ramie type: *a* 8·28, *b* 10·3, *c* 7·89 A., β 84°; (b) coltsfoot type: *a* 8·05, *b* 10·3, *c* 7·98 A., β 89·0°. A further variation suggested [18] has been shown to be due to waxy contamination.[19] Fibre diagnosis by *X*-radiograms is now standard practice for cotton and jute.[20]

Determination of micelle size by low-angle diffuse scattering [31a] and electron microscopy [14] confirm early results by *X*-ray line-broadening (ramie, ~50 × >600 A.; viscose, ~40 × 350 A.).[78a] In this field, intensive studies by the former method have been made by A. Guinier,[21] R. Hosemann,[22] O. Kratky *et al.*,[23] and H. Mark.[31a] The theory of scattering as applied to gases can be applied to other particles; if these are loose-packed and amorphous, each particle scatters with a " particle form factor " akin to that of an atom and its electrons; scattering for the whole is coherent and

[8] *Naturwiss.*, 1943, **31**, 171.
[9] M. v. Ardonne, E. Schiebold, and G. Günther, *Z. Physik*, 1942, **119**, 362.
[10] E. Husemann and A. Carnap, *J. makromol. Chem.*, 1943, **1**, 16.
[11] *E.g.*, O. A. Battista, *Ind. Eng. Chem. Anal.*, 1944, **16**, 351.
[12] J. F. Foster, *Iowa State Coll. J. Sci.*, 1943, **18**, 36.
[13] G. V. Schulz and E. Husemann (with H. J. Löhmann), *Z. physikal. Chem.*, 1942, *B*, **52**, 23, 50.
[14] *E.g.*, D. Beischer, Discussion, *Z. Elektrochem.*, 1940, **46**, 550; A. Hamann, *Kolloid. Z.*, 1942, **100**, 248.
[15] B. Ingleman and K. Siegbahn, *Arkiv Kemi, Min., Geol.*, 1944, *B*, **18**, No. 1.
[16] W. A. Sisson, *Contr. Boyce-Thompson Inst.*, 1941, **12**, 31.
[17] K. H. Meyer and A. van der Wyk, *Z. Elektrochem.*, 1941, **47**, 353.
[18] N. Gralén, S. Berg, and T. Svedberg, *Ber.*, 1942, **75**, 1702.
[19] K. Hess, H. Kiessig, and W. Wergin, *Ber.*, 1943, **76**, 449.
[20] Ann. Rep. Ind. Assoc. Cult. Sci., 1943. [21] *Ann. Physique*, 1939, **12**, 161.
[22] *Z. Elektrochem.*, 1940, **46**, 535; *Z. Physik*, 1939, **113**, 751.
[23] O. Kratky, *Naturwiss.*, 1942, **30**, 542.

additive. For particles of micelle size, this scattering is intense only at very low angles. By comparing the intensity distribution found with that calculable from geometrical systems (*e.g.*, elongated ellipsoids for fibres), size, shape, and orientation of the macrostructures may be determinable. Treatments differ, Hosemann following Guinier with increased precision, Kratky, doubtful of the interference of internal particle structure and close-packing, preferring a statistics of interdistances. Conditions can be improved by suitable swelling agents or inter-micellar deposition of heavy scattering material.[25, 31a] By such means, *n*-paraffin lamellæ have been found 400 A. thick, and distributions for ramie and cellulose triacetate have maxima at 3000, 200 A. respectively (length) and $\ll$400 A. (width).[22] In favourable cases (*e.g.*, colloid sols of spherical chymotrypsin) precision may reach $\pm$3 A.[24]

The crystalline-amorphous ratio (C/A $\equiv \phi$) is important in physical properties of cellulose fibres. Alternative methods of defining " crystalline " and " amorphous " are not necessarily identical, for fringe intermicellar material, especially on stretching, has more than random order though without exact phase relationship. Broadly speaking, the crystalline regions have lower swelling, energy, entropy, little accessibility to dyes and mild chemical reagents, greater compactness and density, and measurements involving these are indicative. Where C $\rightleftharpoons$ A (rubber), ϕ is simply got by *X*-ray diffraction ;[26] where C $\rightleftharpoons$ A (cellulose), calibration may be made from like mixtures, *e.g.*, sugar (C)—sugar glass (A).[27] Measurements of density (rubber), latent heat of fusion (polythene), vapour pressure isotherms (nylon, cellulose) have been employed; swelling anisotropy, magnetic anisotropy, birefringence are applicable. Chemical methods for cellulose include peroxidation [28] and thallation in alkyl ethers.[29] Indications are also derivable by use of the Tuckett equation,[74a] or in certain cases from volume changes above a second-order transition point.[82a]

Structural indications are given well by swelling phenomena in conjunction with *X*-ray analysis. The transition cellulose $\rightleftharpoons$ hydrate-cellulose has been followed by *X*-rays.[30] Swelling by *n*-alkyl primary amines takes place exclusively between the (101) by the creation of O · · H · · N links, D_{101} increasing regularly with length of alkyl chain.[31] By the use of nitrogen pentoxide, important results for the nitrocellulose systems have been obtained by something approaching *X*-ray cinematography ;[32] the nature of

[24] O. Kratky, B. Baule, A. Sekora, and R. Treer, *Kolloid. Z.*, 1942, **98**, 170; O. Kratky and A. Sekora, *Naturwiss.*, 1943, **31**, 46.

[25] O. Kratky and F. Schossberger, *Z. physikal. Chem.*, 1938, *B*, **39**, 1451; O. Kratky, A. Sekora, and R. Treer, *Z. Elektrochem.*, 1942, **48**, 587.

[26] J. E. Field, *J. Appl. Physics*, 1941, **12**, 23.

[27] I. Fankuchen and H. Mark, *Rec. Chem. Progr.*, 1943, July—Oct., 54.

[28] G. Goldfinger, H. Mark, and S. Siggia, *Ind. Eng. Chem.*, 1943, **35**, 1083.

[29] A. G. Assaf, R. H. Haas, and C. B. Purves, *J. Amer. Chem. Soc.*, 1944, **66**, 59.

[30] T. Kubo, *Kolloid. Z.*, 1940, **93**, 338.

[31] W. E. Davis, A. J. Barry, F. C. Peterson, and A. J. King, *J. Amer. Chem. Soc.*, 1943, **65**, 1294.

[32] M. Mathieu, *Compt. rend.*, 1941, **212**, 80.

chain slip and the growth of the di- and tri-nitro-derivatives is followed, while on swelling with acetone gelatiniser, definite lattice structures exist at 1 mol./glucose, 1 mol./O·NO_2, 3 mols./glucose, beyond which the fibre structure breaks down.[33] Similar experiments with series of ketones, esters, and alcoholic nitrates [34] show unidirectional swelling, only *a* of the monoclinic cells varying appreciably. Water is of special structural importance. For cellulose, adsorption isotherms have been determined, and heats of sorption noted.[35] The adsorption curves are 3-stage sigmoid. The heat of adsorption for hydrate-cellulose at 3·5 kg.-cals./mole = twice that for native cellulose (1·69), giving, per mole of water, the heat of adsorption value for hydration of β-sugars and alcohol–water mixtures. The energy difference between normal and superfinely ground sugars equals the heat of solvation, superfine grinding also destroying the lattice.[36] Thus heats of sorption are an index of ϕ in a fibre. For cellulose, water sorption falls into three categories [37]—

(*a*) 1 H_2O per primary OH } interpreted as a unimolecular layer on the
(*b*) 1 H_2O per secondary OH } accessible surface.
(*c*) Collection in capillaries : non-structural.

At stage (*a*), compression of water takes place, presumably by attachment through two hydrogen bonds. The hydrate-cellulose lattice is not distorted. A treatment using the Langmuir and Brunauer–Teller mono- and multi-layer adsorption isotherms is that of J. D. Babbitt.[38] Bound water has also been surveyed by K. C. Blanchard.[39]

An interesting application of specific rotatory power locates the copper in the cuprammonium cellulose complex.[40] By comparison with glucosides, sufficient conditions for similarly enhanced rotations are shown to be free hydroxyls at positions 2 and 3 and substitution at 4.

Fine Structure.—(a) *Alginic acid.* For this seaweed fibre consisting of a succession of β-*d*-mannuronic acid residues,[41] the rhombic cell has *a* 8·60, *b* (fibre axis) 8·72, *c* 10·74 A., space-group $V^3(P2_122_1)$, almost $V^4(P2_12_12_1)$.[42] The cell contains, in addition to 4 residues, ~$4H_2O$ which extend *a* only; for the dry cell, $a = 7{\cdot}75$ A. Using standard bonds and angles and the chair form of glucose ring, two pairs of positions for glucosidic *O*-linking permit a linear chain extension parallel to the fibre axis. One pair gives a period of 4·35 A., hence probably holds for alginic acid (cf. 2 × 4·36) and probably for pectin also (because of the stereochemical relation of side groups

[33] M. Raison and M. Mathieu, *Compt. rend.*, 1941, **212**, 157; G. V. Schulz, *Z. physikal. B*, **52**, 253.
[34] T. Petitpas, Thèse, 1943, Paris.
[35] K. Lauer, R. Döderlein, C. Jäckel, and O. Wilde, *J. makromol. Chem.*, 1943, **1**, 76.
[36] J. Gundermann, *Kolloid. Z.*, 1942, **99**, 142.
[37] A. G. Assaf, R. H. Haas, and C. B. Purves, *J. Amer. Chem. Soc.*, 1944, **66**, 66.
[38] *Canadian J. Res.*, 1942, *A*, **20**, 143.
[39] *Cold Spring Harbor Symp. Q. Biol.*, 1940, **8**, 1.
[40] R. E. Reeves, *Science*, 1944, **99**, 148.
[41] E. L. Hirst, J. K. N. Jones, and W. O. Jones, *J.*, 1939, 1880.
[42] W. T. Astbury (in press).

in α-galacturonic and β-mannuronic residues). A full spatial analysis will be of interest.

(b) *Cellulose.* The second pair of positions gives an extension of 5·18 A., suggesting a normally built structure for cellulose (and chitin). While G. L. Clark's analysis [43] confirmed the Meyer–Misch structure,[44] models show some discrepancies. The Astbury–Davies skeletons for alginic acid and cellulose reveal the possibility of interchange with only slight bond strain,[45] with obvious repercussions on carbohydrate stereochemistry. Other recent modifications have been suggested. P. H. Hermans's model,[46] using standard bonds and angles, provides a similar bent cellobiose radical to fit the fibre period, and does not permit parallelism in successive glucose rings. Chains have alternating polarities in successive layers along *c*; along *b* chain layers are staggered by 3—4 A. F. T. Peirce [47] proposes three modifications of the Meyer–Misch model :

(i) A pyranose ring with perpendicular $\diagup O \diagdown$ valencies and more nearly coplanar C's, though precision analyses on simpler sugars strongly favour the unstrained chair model.

(ii) With the principal plane of the rings in the *ab* plane, the primary alcohol groups can form OH bonds with the pair of secondary alcohol groups of the adjacent parallel chain; an alternative bond with 2 O of the same chain is held to account for the slight departure from rhombic symmetry.

(iii) The pyranose rings are turned out of the *ab* plane of similarly oriented chains towards that containing those of alternate sense, with OH bonding between the two sets of chains instead of within one. This model is claimed to explain better identity period, symmetry, and the facts of regenerated cellulose.

Structures for lignin [48] and pectin [49] have been advanced; the fibre period of the latter is 8·7 A. as with alginic acid. Lignin is a polycapillary (30—20,000 A. diameter) lamellar disperse organophilic mixture.

(c) *Starch.* The two components of starch, amylose and amylopectin, are respectively linear and branched chains. P of amylose and of the inter-branch length of amylopectin are determinable by measurements of the molecular extinction coefficient and λ of peak absorption.[50] Starch chains are more flexible than the cellulose type [51] and configurations vary with treatment.[52] In the fibrous " B " modification, chains are fully extended in a rhombic cell with *a* 16·0, *b* (fibre axis) 10·6, *c* 9·2 A., probable space-group $V^{(1)}$.[52] Butanol-precipitated amylose and the starch–iodine complex,

[43] S. T. Gross and G. L. Clark, *Z. Krist.*, 1938, **99**, 357; *Text. Res.*, 1938, **9**, 7.
[44] K. H. Meyer and L. Misch, *Helv. Chim. Acta*, 1937, **20**, 232.
[45] W. T. Astbury and M. M. Davies, *Nature*, 1944, **154**, 84.
[46] P. H. Hermans, J. de Booys, and C. J. Maan, *Kolloid. Z.*, 1943, **102**, 169.
[47] *Nature*, 1944, **153**, 586.
[48] R. Jodl, *Brennstoff-Chem.*, 1942, **23**, 163, 178; cf. *Ann. Reports*, 1942, **39**, 142.
[49] F. A. Henglein, *J. makromol. Chem.*, 1943, **1**, 121.
[50] R. R. Baldwin, R. S. Bear, and R. E. Rundle, *J. Amer. Chem. Soc.*, 1944, **66**, 84
[51] R. E. Rundle and L. W. Daasch, *ibid.*, 1943, **65**, 2261.
[52] R. E. Rundle, L. Daasch, and D. French, *ibid.*, 1944, **66**, 130.

however, reveal a helical starch chain of pitch and diameter 8 and 13·7 A. respectively, with six glucose residues per turn. The helices are approximately close-packed, alternate helices oppositely alined, both space-groups probably V^4–$P2_12_12_1$.[53] Like the iodine, the butanol is enclosed lengthways inside the helix, absorption optics confirming this.[54] With glycerol as plasticiser, another fibrous form of amylose (period 7·5 A.) is obtained.[51] Polarisation optics differentiate this from the "V" form, and a linear fold rather than a helical chain is indicated.[51] Starch cannot absorb iodine vapour when in the "A" or "B" configuration, but on conversion into the "V" configuration, by alcohol precipitation, iodine is taken up up to 1 I_2 per six glucose rings.[55]

Proteins.—(a) *General.* Despite difficulties in recording, resolving, indexing, and analysing fibre *X*-radiograms of large and protean chains liable to polyphase and cytological structure, mixed crystallisation, polymorphism, disorder of strain, layering, and swelling, giving possibly only local crystallisation of many-parameter elements of uncertain chemical content, structural progress continues by the combined use of all relevant physico-chemical techniques. Structural unity in crystalline and fibrous proteins is now recognised as based on the packing of polypeptide and side-chain elements. D. Wrinch's structural basis [56] is the polypeptide chain mesh itself, with its cyclisations and folding to cage fabrics; its success in interpreting structural protein features and *X*-ray data, *e.g.*, for insulin, has been variously estimated.[57] W. T. Astbury's basis [58] is the packing of side chains, the polypeptide skeleton adopting the configuration permitting close-packing and the presentation of the specific patterns so characteristic of enzyme action. For keratin, a lamellar structure completely reoriented with reference to the fibre axis has been suggested.[59] Recent general analyses are due to M. L. Huggins [60] and D. G. Dervichian.[61] Using principles such as pseudo-hexagonal close-packing, standard bonds and angles, N··H··O bridging, *l*-configuration of residues, and a fibre screw axis (to prevent any asymmetric bending tendency), the former derives conceivable skeleton structures for silk fibroin, β- and α-keratin, and collagen. The favoured α-structure resembles Astbury's and adheres to a 100% intramolecular extension in the α ⟶ β transition. The collagen model consists of linked spirally-coiled polypeptide chains with a pseudo-rhombic cell having *a* 4·5, *b* 5·8, *c* 22 A. Dervichian postulates for proteins a two-dimensional double layer of amino-acids, with polar and non-polar groups on opposite sides of a layer. Polar groups normally form the external surface. Internal structure is imposed by symmetrical

[53] R. E. Rundle and F. C. Edwards, *J. Amer. Chem. Soc.*, 1943, **65**, 2200.
[54] R. R. Baldwin, *Iowa State Coll. J. Sci.*, 1943, **18**, 10.
[55] R. E. Rundle and D. French, *J. Amer. Chem. Soc.*, 1943, **65**, 1707.
[56] *Phil. Mag.*, 1940, **30**, 64; *Proc. Roy. Soc.*, 1937, *A*, **161**, 505; *A*, **160**, 59.
[57] I. Langmuir, *Proc. Physical Soc.*, 1939, **51**, 592; I. Langmuir and D. Wrinch, *ibid.*, p. 613; J. D. Bernal, *ibid.*, p. 618; L. Pauling and C. Niemann, *J. Amer. Chem. Soc.*, 1939, **61**, 1860; D. Wrinch, *ibid.*, 1941, **63**, 330.
[58] *J.*, 1942, 337.
[59] L. W. Janssen, *Protoplasma*, 1939, **33**, 410.
[60] *Chem. Rev.*, 1943, **32**, 195.
[61] *J. Chem. Physics*, 1943, **11**, 236.

hexagonal packing of parallel rod-like side chains. One plate is thus the minimum unit, its component amino-acids necessarily in 2^m3^n proportions. The passage from globular to fibrous state is simply the smectic-nematic transition of liquid crystals.[62] In denaturation, a plate is ruptured and the polypeptide chain, presenting hydrophobic groups also, becomes the dominant element.

Examination of the 2^m3^n rule for the occurrence and distribution of amino-acid protein components continues. The rule, established by ultracentrifuge measurements,[63] follows structurally from the geometry of Wrinch's cages and Dervichian's symmetric packings; support for its spatial regularity is given by analysis of keratin fibre *X*-radiograms.[64] From a wealth of chemical analyses by methods of improved precision,[32a, 65] it is now clear [66] that the rule is followed mostly with high precision, but with equally precise exceptions, which, however, do not necessarily invalidate the rule for component lamellæ. A mathematical analysis [67] of the conditions that permit the periodic chain congruences upheld by M. Bergmann,[68] shows that the numbers 2 and 3 have no special privilege in the general case and, further, that for certain proteins, *if constituted as a single chain*, such a distribution cannot occur. Specific evidence against the full rigidity of the Bergmann–Niemann hypothesis is seen in the observed multiple function of certain residues (serine in fibroin,[69] cystine in keratin [70]) and in the components of partial hydrolysis.[71] Dipeptides so formed often corroborate the polar-nonpolar sequence assumed in various theories.[58, 61] The possibility that protein amino-acids may not be exclusively of one optical configuration [72] does not lessen structure-analytical difficulties.

Denaturation *X*-ray studies [73] continue for their structural interest and as an aid in the production of strong protein fibres with wool-like qualities. Protein complexes are formed with detergents such as alkyl benzenesulphonate [75] or sodium dodecyl sulphate.[76] The detergent (*a*) unfolds globular

[62] *Trans. Faraday Soc.*, 1933, **29**, 881.

[63] T. Svedberg, *Proc. Roy. Soc.*, 1939, *B*, **127**, 1.

[64] I. MacArthur, *Nature*, 1943, **152**, 38; *Wool Rev.*, 1940, 9.

[65] D. Bolling and R. J. Block, *Arch. Biochem.*, 1943, **2**, 93; **3**, 217; H. B Vickery, *Ann. N.Y. Acad. Sci.*, 1941, **41**, 87.

[66] A. C. Chibnall, *Proc. Roy. Soc.*, 1942, *B*, **131**, 136.

[67] A. G. Ogston, *Trans. Faraday Soc.*, 1943, **39**, 151.

[68] M. Bergmann and C. Niemann, *J. Biol. Chem.*, 1937, **118**, 301; 1938, **122**, 577; *Chem. Rev.*, 1938, **22**, 423.

[69] A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, 1943, **37**, 538; S. Blackburn, W. R. Middlebrook, and H. Phillips, *Nature*, 1942, **150**, 57.

[70] W. R. Middlebrook and H. Phillips, *Biochem. J.*, 1942, **36**, 294; W. C. Hess and M. X. Sullivan, *Arch. Biochem.*, 1943, **3**, 53.

[71] A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, 1941, **35**, 1369; 1943, **37**, 92; R. L. M. Synge, *Chem. Rev.*, 1943, **32**, 135.

[72] R. L. M. Synge, *Biochem. J.*, 1944, **38**, 285.

[73] M. Spiegel-Adolf and G. C. Henny, *J. Physical Chem.*, 1941, **45**, 931; 1942, **46**, 581.

[74] F. R. Senti, C. R. Eddy, and T. G. Nutting, *J. Amer. Chem. Soc.*, 1943, **65**, 2473.

[75] H. P. Lundgren, D. W. Elam, and R. A. O'Connell, *J. Biol. Chem.*, 1943, **149**, 183.

[76] F. W. Putnam and H. Neurath, *ibid.*, 1943, **150**, 263.

proteins, (*b*) allows precipitation with a minimum of inorganic ion, and (*c*) prevents crystallisation in drawing the fibres. Conditions for effective fibering are a function of protein, nature and concentration of detergent, temperature, and pH.[77] Egg albumin, edestin, zein, casein, pepsin, lactoglobulin, feather keratin, soybean, and peanut proteins have been used; on removal of salt and detergent the steam-extended fibres develop considerable strength.[74] K. J. Palmer's *X*-ray results for egg albumin are important.[78] The complex shows L (30 A.) of detergent and 4·7 and 10 A. rings of protein; detergent removal leaves the disoriented β-form as in heat-denaturation; 500% extension in steam gives the β-form oriented with the definition and degree of silk. Orientation being here a *chain* not a *micelle* phenomenon explains the difference in strength and orientation from Astbury's films.[79] The facts of electrophoresis and the curious compositions and phase stability are quantitatively explained by structures in which denatured egg albumin forms a polar-apolar monolayer with a covering unimolecular layer of close-packed detergent chains held by their hydrophobic ends, and the Astbury–Pauling 4-plate structure [80] of native egg albumin contains detergent as a parallel bimolecular tail-to-tail layer, held close-packed between the appropriate hydrophobic albumin groups so that detergent chains are unidirectionally parallel to the plates and perpendicular to the stack. Detergent-gelatin complexes show a single detergent chain forest-layer polar-bonded to the basic nitrogen of the protein, followed by a covering reverse layer before peptisation.[81]

The prevalence of intercalation, mixed systems, and mutual stoicheiometric accommodation in organic fibres is due to a similarity in C, N, and O bonds and angles; *e.g.*, the aliphatic chain interdistance (4·6 A. near m. p.) ˙=˙ the backbone of a polypeptide grid (4·65 A.), while the relations of the fibre repeat of the common units of structure (Table) are equally striking :

Component unit.	Increment (*I*).	*n*.	*nI*.
Aliphatic $(CH_2)_2$	2·548 A.	4	10·19 A.
β-Keratin	3·38	3	10·14
Nucleotides	3·34	3	10·02
α-Keratin	5·14	2	10·28
Muscle (normal)	5·13	2	10·26
Cellulose, chitin	5·15	2	10·30
Alginic acid, pectin	4·36	2	8·72
Collagen	2·88	3	8·64
Muscle (L–P)	2·83	3	8·48

Viscose–casein, protein–polyamide, polyamide–ester, polyamide–cellulose, and cellulose–protein systems have industrial importance. L. Pauling [82] has

[77] H. P. Lundgren, *J. Amer. Chem. Soc.*, 1941, **63**, 2854.

[78] K. J. Palmer and J. A. Galvin, *ibid.*, 1943, **65**, 2187; K. J. Palmer, *J. Physical Chem.*, 1944, **48**, 12.

[79] W. T. Astbury, S. Dickinson, and K. Bailey, *Biochem. J.*, 1935, **29**, 2351.

[80] W. T. Astbury, *Nature*, 1936, **137**, 803; L. Pauling, *J. Amer. Chem. Soc.*, 1940, **62**, 2643.

[81] K. G. A. Pankhurst and R. C. M. Smith, *Trans. Faraday Soc.*, 1944, **40**, 565.

[82] L. Pauling and D. H. Campbell, *Science*, 1942, **95**, 440.

used the mutual effect of globulin and denaturant to synthesise *in vitro* antibodies specific to the phenylarsonic group. Chromosomes have not yet yielded to *X*-ray structural analysis.[83] Structural results on lipids, nerve, and plasmosin fibres have been recently reviewed.[84]

Water content and its nature are structurally important in protein fibres. J. R. Katz early showed [85] that water entered between the side chains of the gelatin lattice, the spacing increase being linear up to 35% content. A. Weidinger,[86] using $Co(CoCl_4) \rightleftarrows 2[Co(H_2O)_6]Cl_2$, as indicator for free and bound water, identifies these as intra- and inter-micellar by *X*-rays. In further studies, S. E. Sheppard [87] and O. L Sponsler *et al.*[88] find, in addition to the side-spacing increase, a new fixed spacing at 7·5 A., no change in the fibre-axial 2·8 A., but a fall in the backbone from 4·4 to 3·3 A. The latter authors, from consideration of the amino-acid content of gelatin and the normal water-co-ordination of the hydrophilic groups involved, locate the adsorbed water first on the hydrophilic side chains and later on the peptide links, corroborating this by infra-red spectroscopy. J. B. Speakman's analysis [89] of the wool keratin adsorption isotherm shows similar fractions : (i) α-H_2O (up to 6%) combining exothermically with hydrophilic side-chains without effect on such mechanical features as rigidity, (ii) β_1-H_2O, which associates with the peptide groups up to a 1 : 1 limit and affects mechanical properties, and (iii) β_2-interstitial H_2O. A similar study [90] of protein hydration in general, with a spatial molecular interpretation of the energetics of adsorption layers, has important structural implications. In a favourable instance, admirable use has been made of hydration for phase determinations in Fourier synthesis.[91] As with soaps [92] and viruses,[93] intermicellar water may reach great dimensions in lipid-protein complexes without greatly disturbing regularity of array.[94]

The interpretation, on a molecular structural basis, of elasticity and plasticity in protein fibres is difficult, mainly because of their composite crystalline-amorphous nature and the time effect in mechanical tests. Despite its successes and improved α-form, Astbury's $\alpha \rightleftharpoons \beta$ transition in wool, with its reversible 100% intramolecular extension, is not yet universally accepted. The histological objection has been negatived by H. J. Woods.[95] It has been variously suggested that the transformation is an ordinary gel–sol

[83] J. B. Buck and A. M. Melland, *J. Hered.*, 1942, **33**, 173.
[84] F. O. Schmitt, "Advances in Protein Chemistry," 1944, **1**, 25.
[85] J. R. Katz and J. C. Derksen, *Rec. Trav. chim.*, 1932, **51**, 513.
[86] A. Weidinger and H. Pelser, *ibid.*, 1940, **59**, 64.
[87] *J. Physical Chem.*, 1940, **44**, 185.
[88] O. L. Sponsler, J. D. Bath, and J. W. Ellis, *ibid.*, p. 996.
[89] *Trans. Faraday Soc.*, 1944, **40**, 6.
[90] H. B. Bull. *J. Amer. Chem. Soc.*, 1944, **66**, 1499.
[91] J. Boyes-Watson and M. F. Perutz, *Nature*, 1943, **151**, 714.
[92] K. Hess, W. Philippoff, and H. Kiessig, *Kolloid. Z.*, 1939, **88**, 40.
[93] J. D. Bernal and I. Fankuchen, *J. Gen. Physiol.*, 1941, **25**, 111.
[94] K. J. Palmer, F. O. Schmitt, and E. Chargaff, *J. Cell. Comp. Physiol.*, 1941, **18**, 43; R. S. Bear, K. J. Palmer, and F. O. Schmitt, *ibid.*, 1941, **17**, 355; K. J. Palmer and F. O. Schmitt, *ibid.*, p. 385.
[95] *Proc. Roy. Soc.*, 1938, *A*, **166**, 76.

one,[96] is interpretable statistically like that of vulcanised rubber,[96] cannot give 100% fibre extension unless cross-links are broken,[96, 97] is really irreversible,[96] and can be obtained without fibre extension at all; [97] that proto-fibril and macroscopic fibre (*e.g.*, collagen) need not be elastically parallel; [84] that the intramolecular interpretation fails to account for the importance of water in the transition,[98] the correlation of poor elasticity and high crystallinity,[42a, 96] and the absence of long spacings in the β-form; [42a] and that its biological generality and 3-residue α-fold are disproved by the Picken structure for aged muscle.[99] Astbury's point of view stresses the absence of *long-range reversible* extension in straight chains such as fibroin, the specificity of proteins as compared with rubber, the evidence of optical birefringence and denaturation,[79] the generality of the relation in the keratin-myosin group independent of chemical composition as such,[58] and its thread of biological sequence, as all referring it to a fundamental configurational chain feature, the 100% molecular extension best fitting all the evidence. Apart from further recent *X*-ray work,[42a] the developing chemistry of keratin cross-links,[100, 1] consideration of their relation to elasticity and setting in those regions available to acid dyes,[2] and the remarkable properties of the cuprammonium–keratin complex,[3] promise valuable information on these structure–elasticity relations.

(b) *Specific. Fibroin.* M. Bergmann's analytical figures supporting the 2^m3^n distribution rule have undergone review,[71b] and E. Abderhalden [4] maintains a diketopiperazine structure. K. H. Meyer [5] rejects tyrosine from the crystalline region and favours there a regular alternation of alanine and glycine (with 1 serine replacing 1 alanine in 12) to fit the Kratky–Kuriyama cell,[6] which may, however, be a simple fractional relation of the real one. R. Brill [1b] holds that the cell structure is like that of the polyamides, with an extension of *b* to accommodate side chains, and lateral connection of the zigzag chains in the *ac* plane by N · · H · · O. Preliminary results of a recent study [7] suggest chains (*l*, 1300 A.; *M*, 33,000) mainly of regular periodicity, but with two symmetrically-set regions containing the four proline residues in a tyrosine-rich section. Specific structures based on chain folds at 6-co-ordinated copper atoms ($2H_2O$, 2 prol., 1 tyr., 1 other) are proposed for the chains dissolved in cupriethylenediamine (1 Cu to 2 peptide N).

[96] H. B. Bull and M. Gutman, *J. Amer. Chem. Soc.*, 1944, **66**, 1253.
[97] W. Harrison, *Chem. and Ind.*, 1941, 558.
[98] H. J. Woods, *Proc. Leeds Phil. Soc.*, 1940, **3**, 577.
[99] W. Lotmar and L. E. R. Picken, *Helv. Chim. Acta*, 1942, **25**, 538.
[100] T. Barr and J. B. Speakman, *J. Soc. Dyers Col.*, 1944, **60**, 335; J. L. Stoves, *Trans. Faraday Soc.*, 1942, **38**, 254, 501; 1943, **39**, 294, 301; M. Harris *et al.*, *J. Res. Nat. Bur. Stand.*, 1941, **27**, 89.
[1] M. Harris, L. R. Mizell, and L. Foort, *ibid.*, 1942, **29**, 73.
[2] G. H. Elliott and J. B. Speakman, *J. Soc. Dyers and Col.*, 1943, **59**, 124
[3] C. S. Whewell and H. J. Woods, *Nature*, 1944, **154**, 546.
[4] *Z. physiol. Chem.*, 1940, **265**, 23.
[5] K. H. Meyer, M. Fuld, and O. Klemm, *Helv. Chim. Acta*, 1940, **23**, 1441.
[6] O. Kratky and S. Kuriyama, *Z. physikal. Chem.*, 1931, *B*, **11**, 363.
[7] D. Coleman and F. O. Howitt, *Nature*, 1945, **155**, 78.

Fibrin. This insoluble fibrous form of fibrinogen is now shown to be a member of the keratin–myosin group.[8] Normally α-form, the β-form is produced by stretching or lateral pressure. The mechanical properties have been considered on the basis of a mesh structure.[9]

Keratin. EM's [10] confirm the difference in disorder between scale and cortex, proto-fibrils of the former showing a minimum diameter of ~1000 A. without evidence of fine structure. Mercury and water at 80° hydrolyse preferentially the extra-micellar regions of wool (to 48%), no changes showing in the α- or β-pattern, stretched or unstretched.[11] Acidic reagents and hydrogen peroxide produce a degraded structure, held by E. Elöd [12] to be a d-keratin distinct from the β-form. I. MacArthur's precision recording [64b] of the super-structure of porcupine quill yields a very complete spectrum with a fibre period of either 658 or 198 A. (ratio 10/3). The first alternative he shows to contain, with its strong sub-orders, 2^m3^n numbers of amino-acid residue lengths, and suggests a multi-ladder structure with spatial regularities according with much of the Bergmann–Niemann theory; this interpretation is adopted by Astbury.[58b, 13] The smaller figure is preferred by R. S. Bear [14] on the basis of similar *X*-ray measurements. Positions of constituent amino-acid residues have not yet been located in complete accordance with the intensity distribution. R. S. Bear's feather keratin period [14] (95 A.) scarcely includes the full spectrum. A less compact α-keratin structure containing 2 not 3 residues per 5·14 A. period has been suggested.[60b, 96b, 99b]

Myosin. The nature of the muscle proteins, of which myosin is the chief, has been recently reviewed.[15] By ultracentrifuge analysis, two long monodisperse rod-shaped myosin proteins are found [16] and to these the diffractions of muscle are attributed. EM's show protofibrils down to 50—100 A. thick.[17] These have a regular banded structure,[18] especially on treatment with osmic acid,[19] and of mean period 360 A.[19] Long *X*-ray spacings by Bear reveal a regular periodicity of 726 A. up to at least the 40th order, with characteristic intensification at 5 m intervals.[20] Such complementary use of EM and *X*-ray methods is a promising one in protein fibre analysis. On the grounds of the relation of the 5·1 A. period of muscle to this 726 A. spacing, MacArthur's extension of possible 2^m3^n periodicities to muscle [64b] is rejected.

[8] K. Bailey, W. T. Astbury, and K. M. Rudall, *Nature*, 1943, **151**, 716.

[9] U. Ebbecke, *Kolloid. Z.*, 1940, **91**, 134.

[10] H. Zahn, *Textilber.*, 1942, **23**, 157; C. W. Hock and H. F. McMurdie, *Amer. Dyes Repr.*, 1943, **32**, 433, 451.

[11] E. Elöd, H. Nowotny, and H. Zahn, *Textilber.*, 1940, **21**, 385.

[12] *Idem, ibid.*, p. 617.

[13] "Advances in Enzymology," 1943, **3**, 63.

[14] *J. Amer. Chem. Soc.*, 1943, **65**, 1784.

[15] H. B. Bailey, "Advances in Protein Chemistry," 1944, **1**, 289; E. Wöhlisch *Kolloid. Z.*, 1941, **96**, 261.

[16] G. Schramm and H. H. Weber, *ibid.*, 1942, **100**, 242.

[17] M. v. Ardenne and H. H. Weber, *ibid.*, 1941, **97**, 322.

[18] F. Sjöstrand, *Nature*, 1943, **151**, 725.

[19] M. A. Jakus, C. E. Hall, and F. O. Schmitt, *J. Amer. Chem. Soc.*, 1944, **66**, 313.

[20] *Ibid.*, p. 2043.

But, its exact origin apart, this 726 A. spacing is just the 128th multiple ($2^{7}3^{0}$ as for α-keratin) of the *other* muscle fibre period, R. O. Herzog's early result [21] having now been confirmed with the precision of a ramie photograph for an aged preparation.[99b] Recording conditions are critical, new preparations giving only the usual α-keratin structure. From 18 reflections, W. Lotmar and L. E. R. Picken derive the monoclinic cell *a* 11·70, *b* (fibre axis) 5·65, *c* 9·85 A., β 73° 30′, with four amino-acid residues per cell if density = 1·27. The probable space-group C_2^2 affords the dyad screw axis along *b* desired by M. L. Huggins.[60b] A statistical distribution of particular amino-acids is postulated. A structure is found in which *cis*-type peptide residues aline with a fibre-axial extension of 2·83 A., and are laterally bonded by N · · H · · O links. That the maximum intramolecular extension of 25% is insufficient to explain the elastic range observed is met by allocating residual extension to the amorphous regions. The *cis*-formation and axial residue length recall Astbury's collagen form; [22] the normal myosin structure (period 5·1 A.) is held to represent 2 not 3 residues. The crystallite size in the L–P structure must be ≫ the diameter of the fibre bundles (estimated at ~100 A. from the peak of low-angle scattering [23] by O. Kratky), and weak equatorial spacings observed at 33, 42, and 66 A. are attributed [23] to regular side-spacings, though similar values found in nerve fibres have been referred to persistent tissue lipids.[84b]

Collagen. Gelatin. The diketopiperazines found in gelatin hydrolysates may be attributable to artefacts from dipeptides.[24] The macrostructure has been examined by *X*-rays by O. Kratky and A. Sekora [25] and by R. S. Bear.[26] Both find in collagen many orders of a fibre-axial period of 642 A., absent in gelatin. The period varies from 680 to 615 A. according as swelling or tanning procedures are applied, without any corresponding variation in the dominant 2·86 A. period. Thus the lattice and superlattice may have no close common origin. The large period is not removed by swelling in acid or alkali and redrying, but disappears on thermal or chemical shortening of the fibre. The alternation of intensities in odd and even orders when wet, disappears on drying. That the macrostructure seems more a function of protofibril than lattice is confirmed by EM. The 644 A. period so found [27] with possible 160 A. sub-peaks, can be greatly extended by tension, which also alters the ratio of the lengths of dark and light bands. Further elucidation of the nature of this banded superstructure will be of the greatest interest.

Viruses. EM's [28] have confirmed *X*-ray findings. No further analysis has been presented of the wide-angle pattern of tobacco mosaic virus, which seems based on rhombohedral units, though further *X*-ray data indicate a lower

[21] R. O. Herzog and W. Jancke, *Naturwiss.*, 1926, **14**, 1223.
[23] *J. Int. Soc. Leather Trades Chemists*, 1940, **24**, 69.
[23] O. Kratky, A. Sekora, and H. H. Weber, *Naturwiss.*, 1943, **31**, 91.
[24] A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, 1943, **37**, 92.
[25] *J. makromol. Chem.*, 1943, **1**, 113.
[26] *J. Amer. Chem. Soc.*, 1944, **66**, 1297.
[27] F. O. Schmitt, C. E. Hall, and M. A. Jakus, *J. Cell. Comp. Physiol.*, 1942, **20**, 11.
[28] W. M. Stanley, *J. Biol. Chem.*, 1942, **146**, 25.

molecular weight than R. B. Corey's.[29] Optical analysis of solutions under pressure[30] suggests that the ribonucleic acid and protein frameworks combine so that the planes of the purine and pyrimidine rings are mainly parallel to each other and to the plane of the indole ring of tryptophan, and probably perpendicular to the long molecular axis. I. MacA.

vi. *Electron Microscopy.*

The electron microscope is one of the new tools available for the study of the fine structure of matter. Although the fundamental identity of geometrical optics and the dynamics of a particle was known to Hamilton a century ago, the idea of using electrons instead of light in a microscope is a very recent development. Looking back, it now seems curious that the discovery of the electron in 1896 was not very quickly followed by the invention of electron-optics. The knowledge of Maxwell's electromagnetic equations and of Hamilton's optical and dynamical theories, together with the existence of the charged particle, provided all the essential features for the inception of the idea of electron-optics. Nearly 30 years passed before de Broglie, Thomson, Davisson, and Germer showed that electrons, like *X*-rays, are diffracted by crystals just as light is diffracted by a ruled grating. Even the discovery that the motion of an electron is governed by a wave did not lead immediately to the idea of using electrons in a microscope. The first step towards the practical realisation of the electron microscope may probably be recognised in a paper by A. Busch [1] in 1926, in which he showed that electrons could be focused by electrostatic and magnetic fields, just as light is focused by a lens. It is an interesting fact that, although the science of light developed from the ray treatment to the wave theory with all its consequences—interference, diffraction, polarisation—yet with electrons the historical sequence is reversed. Following the period in which they were treated as massive particles, which is analogous to the idea of the photon, the diffraction of electrons, *i.e.*, that aspect of their behaviour which is attributable to a wave, received attention whereas their treatment as rays has only been fully studied in the last ten years or so.

The arrival of the electron microscope probably owes something to developments in many branches of scientific investigation which created a demand for such an instrument. The study of the fine structure of matter received an enormous impetus from the application of the technique of *X*-ray crystal analysis, but the step from magnitudes that were optically visible to the knowledge of atomic spacings and arrangements was so large that a gap was left. The limit of resolution of the optical microscope may be set, in round numbers, at about 10^{-5} cm. as a lower limit. Particles smaller than this may be seen as diffraction discs when suitably illuminated but their

[29] C. G. Vinson, D. K. McReynolds, and N. S. Gingrich, *Missouri Agr. Exp. Stn. Res. Bull.*, 1939, No. 297, 11 pp.

[30] A. Butenandt, H. Friedrich-Freksa, S. Hartwig, and G. Scheibe, *Z. physiol. Chem.*, 1942, **274**, 276.

[1] *Ann. Physik*, 1926, **81**, 974.

size and shape will not be determinable. *X*-Ray diffraction carries us at one step down to orders of magnitude of about 10^{-8} cm., but the method is not very suitable for telling us much about structural detail in the region of 10^{-6} cm. It is here that we may hope the electron microscope will step in and show us a world not accessible at all to light waves and difficult of access by *X*-ray methods.

It is perhaps worth while briefly considering the fields open to inspection by the three methods—light, electrons, and *X*-rays. The following table

		cm.
Limit of unaided vision		10^{-2}
Bacteria		$10^{-4} = 1\ \mu$.
Wave-length of light	red	8×10^{-5}
	violet	4×10^{-5}
	ultra-violet	2×10^{-5}
Viruses, protein molecules, smoke particles, oxide films		10^{-6}
Inter-atomic distances in solids		3×10^{-8}
Wave-length of *X*-rays		$10^{-8} = 1$ A.
Wave-length of 60-KV. electrons		5×10^{-10}

shows a few of the magnitudes of small objects together with the wave-lengths of light, *X*-rays, and electrons. There are, of course, *X*-rays of wave-lengths other than 10^{-8} cm., but for crystal analysis the wave-lengths used are commonly about $1{\cdot}5—2{\cdot}2 \times 10^{-8}$ cm. The *K* characteristic radiations of copper, iron, chromium, and cobalt fall in this range.

The range of magnitudes open to inspection by light is limited at the lower end by the wave-length of light itself. The figure quoted above, 10^{-5} cm., is one-fifth of the wave-length and is probably optimistic. It will be seen that there is a wealth of material, organic and inorganic, below this limit the structure of which cannot be ascertained by optical means. Other things being equal, the limit to the resolving power of *X*-ray or electron microscopes would be the same, *i.e.*, one-fifth of the wave-length. Looking at the table, we see that the *X*-rays used in crystal analysis have a sufficiently short wave-length to enable us to see atoms—always, of course, subject to the above specified condition " other things being equal." The difficulty with *X*-rays is that we have no lenses to bend and focus them in the way that light is focused by the objective and eye lenses of a microscope. All we can do is to record a diffraction pattern and then send out for a mathematician who, given sufficient data, can see with his mind's eye and will produce an electron map of the crystal for us. The resolving power is, in fact, just about the same fraction of a wave-length as in the case of light. The synthesis carried out by the mathematician can be effected by an optical device due to Sir W. Lawrence Bragg,[2] which he has called an *X*-ray microscope.

With electrons the case is somewhat different. The wave-length is extremely short; it is less than that of light by a factor of 10^{-5}; but we cannot yet make use of this enormous potential increase of resolving power. Unlike *X*-rays, but like light, electrons can be deflected from their straight

[2] *Nature*, 1939, **143**, 678; 1942, **149**, 470.

paths—not by material lenses, which would stop an electron beam completely, but by magnetic or electrostatic fields. These electron lenses suffer in an exaggerated degree from the defect known in optics as spherical aberration. There are other defects of the image but this seems to be the most important one. Up to the present, electron lenses free from this defect have not been produced and to minimise its consequences recourse has been had to the device of narrowing the aperture of the lens and using an extremely fine pencil of electrons to illuminate the specimen. The net result is that the resolving power obtained in practice is about 50 A., though in favourable circumstances it seems that a figure of 20 A. may be attained. This means that the resolving power is about 100 times greater than that of an optical microscope.

The growth of electron microscopes from small experimental laboratory models to the type of commercial product which is now available (or would be but for the war) has been fairly rapid and has gone on simultaneously and independently in this country, America, and Germany. Here, L. C. Martin,[3] in collaboration with Metropolitan Vickers, produced an experimental model. Burton,[4] and his collaborators,[5] were at work in Toronto and demonstrated clearly the practical possibilities of high-resolution electron microscopy. In Germany, both Siemens and the A.E.G. had investigators at work and just before the war the former firm had a model for sale, but so far as the Reporter is aware none was imported into this country. The A.E.G. instrument was of the electrostatic type, *i.e.*, the electron lenses were electrostatic fields. This type is stated to be rather simpler in design and construction than the magnetic type, in which the lenses are magnetic fields produced by circular coils carrying a current. Published photographs by H. Mahl [6] and others at magnifications up to 10,000 are certainly of good quality. The magnetic instruments appear to be capable of rather higher magnifying power but the stabilisation of the electric supplies presents a difficult problem.

The Toronto instrument has been developed commercially by the Radio Corporation of America, and several of these instruments are now in service in this country. The lenses are magnetic and their functions are closely analogous to the lenses of the optical microscope. An image of the electron source, a hot tungsten filament, is focused on the specimen by a condenser lens; an objective lens produces a magnified image of the specimen and this image is subjected to a further stage of magnification by a projector lens. The final image at from 2000 to 30,000 diameters is viewed on a fluorescent screen and can be recorded photographically. Usually for high magnification work an electron magnification of about 10,000 is used, followed by

[3] L. C. Martin, R. V. Whelpton, and D. H. Parnum, *J. Sci. Instr.*, 1937, **14**, 14.

[4] E. F. Burton and W. H. Kohl, "The Electron Microscope," Reinhold Corp., New York, 1942.

[5] E. F. Burton, J. Hillier, and A. Prebus, *Physical Rev.*, 1939, **56**, 1171; A. Prebus and J. Hillier, *Canadian J. Res.*, 1939, *A*, **17**, 49.

[6] *Z. tech. Physik*, 1939, **20**, 316.

optical enlargement up to 10 times. These R.C.A. instruments are arranged so that specimen and photographic plate can be introduced into the vacuum through air locks; these operations can be carried out quickly without destroying the vacuum in the body of the microscope column.

Recently the R.C.A.[7] have described a simpler and smaller model, also of the magnetic type. The G.E.C. of America [8] have produced a simplified electrostatic model. Both firms have realised that there will probably be a demand for a cheaper and smaller instrument in industry, hospitals, and universities, with not quite the same high resolving power, but still capable of giving results much in advance of anything obtainable optically. The G.E.C. model is particularly simple. The electron magnification is about 500, and the image on the fluorescent screen is photographed outside the vacuum, a further stage of optical magnification up to a total of about 5000 being possible. These smaller instruments would certainly appear to have some points in their favour for routine work where the highest resolving power is not required.

The instruments mentioned above are all of the " optical " type in the sense that there is a very close analogy between the function of the electron lenses and the lenses of the optical microscope. There are several other devices, which may be called microscopes since they are intended to reveal fine structure, two of which deserve mention. M. Benjamin and R. O. Jenkins [9] have described a method of investigating the auto-electronic emission from fine metallic points which produces a very high magnification. In principle, the apparatus consisted of a very small spherical tip of metal at the centre of a large evacuated spherical vessel coated internally with a thin layer of fluorescent material. A difference of potential was maintained between the metal point and the sphere sufficient to drag electrons from the metal. The electrons starting with negligible velocity follow the straight lines of electrostatic force, and an image of the point is projected on to the surface of the containing vessel. The magnification is the ratio of the radii of the containing vessel and the metal tip. V. K. Zworykin,[10] J. Hillier, and R. L. Snyder [10] have described an electron microscope of a novel kind suitable for the examination of opaque, massive specimens, such as metals. The idea is to use the electron lenses to produce a very fine beam or pencil of electrons. This is moved over the area to be examined, and the secondary electrons, released from the bombarded surface, are accelerated on to a fluorescent screen where they produce light, the intensity of which depends on the number of secondaries and therefore on the nature of the very small area subject to bombardment. The light fluctuations from the fluorescent screen are converted into electrical impulses by a photo-electric cell, and these impulses after amplification are recorded on a drum where a magnified picture of the surface is built up. The instrument will produce magnified images up to some 5000 diameters.

[7] V. K. Zworykin and J. Hillier, *J. Appl. Physics*, 1943, **14**, 658.
[8] C. H. Bachman and S. Ramo, *ibid.*, p. 155. [9] *Proc. Roy. Soc.*, 1940, *A*, **176**, 262.
[10] *Amer. Soc. Testing Mat.*, Aug., 1944; C. J. Overbeck, *J. Sci. Instr.*, 1944, **21**, 1.

Applications.—(a) *Biological.* One of the most interesting fields to which electron microscopy is being applied is in microbiology. There are obviously serious drawbacks to be overcome; the necessity of placing the specimen in a vacuum and bombarding it with high-speed electrons is to be taken into account, and due care must be exercised in ensuring that the results are not affected by changes induced by drying of the material. Notwithstanding the perishable nature of the material and the manifest ill-treatment to which it is subjected, there seem to be grounds for believing that these drawbacks are not in fact so serious as might at first sight appear. Bacteria, viruses, and tissue seem to withstand a reasonable amount of bombardment in a vacuum without undergoing any progressive change in appearance. I. M. Abraham and J. W. McBain [11] have described a method of enclosing the specimen in a cell which is thin enough to allow the electrons to pass without much scattering and yet strong enough to enclose the specimen in a film of water. Applications of the electron microscope to biological problems have been recently reviewed by G. E. Donovan,[12] who gives a list of recent papers on this aspect of the matter. There seems to be a wide field for investigation, and it is being quickly explored.

(b) *Inorganic.* Under this heading there appear to be at present two main uses for the electron microscope. The first is the determination of grain size and shape in smokes, powders, clays, etc. The great opacity of small grains to electrons makes their use particularly apt; particles of colloidal dimensions, 100 A. in diameter, can be detected, and the thickness of material which crystallises in flakes, can be estimated. As examples of this type of investigation attention may be directed to a paper by T. Marx and G. Wehner [13] on the shape and size of particles of $Mg(OH)_2$, which appear to be flakes about 100 A. thick. Carbon soot from different sources has been examined by various workers,[14, 15] and at a recent conference called by the British Coal Utilization Research Association a short account of the application of the electron microscope to the study of coal was given.[16] The changes in shape and size of particles in the transformation of γ-FeO·OH into γ-Fe_2O_3 and α-Fe_2O_3 have been studied by R. Fricke, T. Schoon, and W. Schroder.[17] Mention must be made of the work of C. E. Hall and A. L. Schoen [18] at the Eastman Research Laboratories on the structure of silver bromide grains in photographic emulsions. The change in the bromide crystals during exposure to the electron beam is recorded and the production of filaments of silver in the development of the exposed bromide is shown.

The second type of problem in the inorganic world is metallurgical.

[11] *J. Appl. Physics*, 1944, **15**, 607.
[12] *Nature*, 1944, **154**, 356.
[13] *Kolloid-Z.*, 1943, **105**, 226.
[14] U. Hofman, A. Ragoss, and F. Sinkel, *ibid.*, 1941, **96**, 231.
[15] M. von Ardenne and U. Hofman, *Z. physikal. Chem.*, 1941, *B*, **50**, 1.
[16] *Ibid.*, p. 13.
[17] G. D. Preston and F. W. Cuckow, 1943 Conference on the Ultra-fine Structure of Coal and Cokes, B.C.U.R.A.
[18] *J. Opt. Soc. Amer.*, 1941, **31**, 281.

The examination of an etched metal surface requires a special technique [19] which appears likely to yield valuable results. To examine the surface, it is reproduced in the form of a thin cast or replica in a film of some suitable material such as nitrocellulose or " formvar " (polyvinyl formal). A dilute solution of one of these materials is allowed to dry on the surface of the metal and is then detached and used in the electron microscope as a specimen. Surface irregularities developed on the metal by etching are reproduced in the film as variations of thickness which cause differences in the intensity of the transmitted electron beam. A highly magnified image of the surface can thus be obtained. In certain cases a thin oxide film may be detached from the metal and used as the specimen in the same way; this technique has been used successfully with aluminium alloys. The variation of surface elevation and the thickness of small particles have also been investigated by R. D. Heidenreich and L. A. Matheson,[20] using a stereoscopic method. Changes in elevation and thicknesses of 150 A. can be measured.

(c) *Organic.* The intensive development of synthetic polymers in recent years has focused attention on the large molecular aggregates in these substances. Some attempts have been made to examine these structures by means of the electron microscope. Photomicrographs of polyoxymethylene [21] crystals degraded by acids and alkalis have been published. The same paper contains some observations on chemical and bacterial degradation of cellulose fibrils.

The above very brief summary of the uses to which the electron microscope has been put shows that up to the present the work carried out has been of an exploratory nature. Both the type of problem suitable for investigation and the technique of obtaining specimens in a form suitable for examination must inevitably first be found. At the same time the observations have to be correlated with existing knowledge obtained by optical methods on the one hand and by *X*-ray and electron diffraction on the other. Progress is undoubtedly being made quickly and it may be hoped that with the advent of simpler instruments electron microscopes will be as commonly found in hospital, industrial, and university laboratories as are their optical prototypes. A very valuable bibliography of this rapidly growing subject has been compiled by C. Marton and S. Sass.[22]

G. D. P.

A. E. Alexander.
R. M. Barrer.
C. N. Hinshelwood.
I. MacArthur.
G. D. Preston.
J. M. Robertson.

[19] V. J. Schaefer, *Physical Rev.*, 1942, **62**, 495; *J. Appl. Physics*, 1942, **13**, 427; R. D. Heidenreich and V. G. Peck, *ibid.*, 1943, **14**, 23.

[20] *Ibid.*, 1944, **15**, 423.

[21] M. Staudinger, *Chem. Ztg.*, 1943, **67**, 316.

[22] *J. Appl. Physics*, 1943, **14**, 522; 1944, **15**, 575.

INORGANIC CHEMISTRY.

THE task of reviewing progress in Inorganic Chemistry during the past year has been made very difficult by war conditions, which have no doubt prevented publication of the more outstanding recent advances. The limited availability of numerous continental publications has also restricted the field under review.

Section 1 of the present Report comprises a general survey in which an attempt has been made to correlate the salient developments of the past year. In Sections 2 and 3 the chemistry of gallium and germanium has been reviewed; these elements seem to have been studied along parallel lines ever since both were predicted in the classical work of Mendeléef, and numerous additions to their chemistry appear in the literature of the past few years.

There is at present an evident lack of modern British reference books on Inorganic Chemistry, and of a journal in the English language primarily devoted to inorganic topics. It is to be hoped that attention will be given to these matters when more favourable conditions return.

1. GENERAL.

Separation and Use of Isotopes.—Continued interest in the preparation and use of separated isotopes is evident, and the varied nature of the work recently reported augurs well for further rapid development of this fundamental and widely-useful field of chemistry. The chemical exchange method largely developed by H. C. Urey [1] and the thermal diffusion method of K. Clusius and G. Dickel [2] still provide the most successful means of separating isotopes in reasonably high yield. Nitrogen preparations containing 6·0% of ^{15}N have been obtained by chemical exchange,[3] the reaction employed being $^{15}NH_4$ (liquid) + $^{14}NH_3$ (gas) $\rightleftharpoons$ $^{14}NH_4$ (liquid) + $^{15}NH_3$ (gas). In this case exchange occurs between 60% ammonium nitrate solution and ammonia gas at 90 mm. pressure in packed towers, through which gas and solution pass in the same direction. The separation of oxygen and carbon isotopes by means of the reactions CO_2 (dissolved) + $H_2O \rightleftharpoons H_2CO_3$ and CO_2 (dissolved) + $OH' \rightleftharpoons HCO_3'$ has also been examined, and a strong catalytic effect of solid surfaces on the exchange process detected.[4] In a brief theoretical study [5] of the type of exchange reaction favoured by Urey, a basis has been secured for predicting the manner in which heavy and light isotopes of the same species may be distributed between the gaseous and the liquid phase.

[1] Cf. *Ann. Reports*, 1941, **38**, 83. [2] Cf. *ibid.*, 1940, **37**, 153.
[3] K. Clusius and E. Becker, *Z. physikal. Chem.*, 1943, *A*, **193**, 64.
[4] A. F. Reid and H. C. Urey, *J. Chem. Physics*, 1943, **11**, 403.
[5] L. Waldmann, *Naturwiss.*, 1943, **31**, 205.

The outstanding recent success of the Clusius–Dickel method is the separation of substantially pure samples of $^{18}O_2$ and $^{15}N^{14}N$.[6] This was accomplished with six separation tube units with an aggregate length of 82 m. In the case of oxygen the equilibrium $2^{16}O^{18}O \rightleftharpoons {}^{16}O_2 + {}^{18}O_2$ is set up in the vicinity of the hot wire, and $^{18}O_2$ can be concentrated at the "heavy" end of the system without difficulty. With nitrogen, however, rearrangement of $^{15}N^{14}N$ molecules to $^{15}N_2$ and $^{14}N_2$ does not occur in the tube under normal conditions, and the "heavy" product is $^{15}N^{14}N$; if a suitable catalyst is incorporated in the tube, separation of $^{15}N_2$ becomes possible.

Calculations have recently been made of the energy required to separate the uranium isotope ^{235}U by thermal diffusion of the volatile uranium hexafluoride.[7] The interesting conclusion is reached that the total energy needed for the separation would be 40—80% of the energy yielded by subsequent fission of the ^{235}U nuclei. If this estimate is verified, and if no more efficient method of separating the light isotope can be formed, the use of uranium as a source of nuclear energy does not offer the possibilities often supposed previously.

The separation of oxygen isotopes by distillation of water in a series of three 25-foot fractionating columns has recently been studied.[8] Distillation for 120 days afforded products containing in all 23 g. of ^{18}O in excess of normal; these included 150 ml. of water enriched 6·5-fold in ^{18}O, and 2·7-fold in ^{17}O.

Non-metallic Halides and Related Compounds.—Attention has recently been given to a wide range of volatile mixed halides and related derivatives of the non-metals. Details have now been given [9] of the preparation of all three chloro*iso*cyanates of silicon, $SiCl_3NCO$, $SiCl_2(NCO)_2$, and $SiCl(NCO)_3$, by interaction of silicon tetrachloride and silicon tetra*iso*cyanate at temperatures ranging from 135° to 600°, and by reaction of silver *iso*cyanate with excess of silicon tetrachloride in solution in organic solvents. The latter reaction apparently affords no $SiCl(NCO)_3$; the former gives all three compounds, any mixture or pure compound in the range $SiCl_4$–$Si(NCO)_4$ undergoing "random rearrangement" at elevated temperatures, in the absence of a catalyst. Even at room temperature, $SiCl_2(NCO)_2$ rearranges to the extent of several units % in four months, a fact which indicates that rearrangement reactions must be taken into account wherever the purity of a compound of this general type is in question after storage.

The ease with which these "mixed" compounds undergo rearrangement reactions has also hampered physicochemical measurements intended to show the effects of progressive substitution; it is on record [10] that the compounds PCl_2NCO, $POCl_2SCN$, and $SiCl_3SCN$ have been obtained, but

[6] K. Clusius, G. Dickel, and E. Becker, *Naturwiss.*, 1943, **31**, 210.
[7] A. E. Brodski, *Acta Physicochim. U.R.S.S.*, 1942, **17**, 224.
[8] H. G. Thode, S. R. Smith, and F. O. Walkling, *Canadian J. Res.*, 1944, **22**, 127.
[9] H. H. Anderson, *J. Amer. Chem. Soc.*, 1944, **66**, 934.
[10] H. H. Anderson, unpublished data; cf. ref. (11).

rearrangements have apparently prevented isolation of the other members of these replacement series by fractional distillation. The stability of the silicon–oxygen bond suggests that mixed derivatives containing oxygen directly bound to silicon might rearrange less readily, and this has been confirmed in the case of the silicon methoxy*iso*cyanates. All three of these compounds, *viz.*, $Si(OMe)(NCO)_3$, $Si(OMe)_2(NCO)_2$, and $Si(OMe)_3NCO$, have been isolated [11] by fractional distillation of the products of the reaction between silicon *iso*cyanate and methyl alcohol at room temperature. These compounds evidently rearrange very slowly, the reaction of the dimethoxydi-*iso*cyanate in a tube at 600° being too slow for convenient study.

Reactions of silicochloroform and hexachlorodisilane with silver cyanate, investigated in attempts to prepare corresponding silicon *iso*cyanates, gave silicon *iso*cyanates and cyanate together with free silicon. Use of lead cyanate with hexachlorodisilane gave a colourless product which could not be distilled without decomposition, and this may contain $Si_2(NCO)_6$.[12]

Rearrangement reactions have also been examined in several other instances covering the halides of carbon, silicon, germanium, and stannic tin; [13] in all these cases the chlorobromides appear to coexist in random distribution, the rearrangements occurring with increasing facility on passing from carbon to tin. In the CCl_4–CBr_4 system appreciable rearrangement requires the presence of moist aluminium chloride as a catalyst, the change to random distribution then being complete after 7 hours at 170°. Silicon chlorobromides (and chloroiodides) rearrange on passage through a tube at 600°, and the products are separable by fractional distillation. The germanium and stannic compounds appear to rearrange so readily that separation of the components by distillation is not possible and the existence of randomly-distributed mixed halides can be inferred only from the character of their "distillation curves," obtained by plotting boiling point against total volume of distillate collected. Evidence for the existence of a new germanium chlorobromide, $GeCl_3Br$, b. p. 112° (approx.), was obtained.

Introduction of fluorine into non-metal halides by conventional methods has been further studied. The partly fluorinated silanes, $SiHF_3$, SiH_2F_2, and SiH_3F, the last two of which are new, have been prepared by the action of antimony trifluoride, in presence of antimony pentachloride, on the corresponding chlorides.[14] The products have anomalously high boiling points, silylene fluoride (b. p. — 77·8°) being less volatile than silicon tetrafluoride; the Trouton constants are high, indicating considerable association in the liquid phase. All three compounds undergo slow disproportionation, even at room temperature, to monosilane and silicon tetrafluoride, this tendency being most marked with silicofluoroform and silyl fluoride.

The action of zinc fluoride on mono- and di-ethyl- and -phenyl-silicon chlorides has also been shown to yield the corresponding fluorides.[15] These

[11] G. S. Forbes and H. H. Anderson, *J. Amer. Chem. Soc.*, 1944, **66**, 1703.
[12] *Idem, ibid.*, p. 1706. [13] *Idem, ibid.*, p. 931.
[14] H. J. Emeléus and A. G. Maddock, *J.*, 1944, 293.
[15] H. J. Emeléus and C. J. Wilkins, *J.*, 1944, 454.

are also obtained by the action of hydrogen fluoride on the appropriate siliconic acids, $(R{\cdot}SiO_2H)_x$, and silicones, $(SiR_2O)_x$, (affording the mono- and di-alkyl compounds, respectively). The reactivity of these alkylsilicon fluorides falls sharply as alklyl substitution proceeds; monoethylsilicon fluoride is immediately (although incompletely) hydrolysed by water at room temperature, whereas the triethyl compound resists hydrolysis strongly.[16] Attempts to prepare phenylsilicon fluorochlorides by treatment of the trichloride with a deficit of zinc fluoride were unsuccessful.

Reaction of thiophosphoryl bromide, $PSBr_3$, with antimony trifluoride, in the absence of a catalyst, affords the corresponding fluoride, PSF_3, and the two bromofluorides, PSF_2Br and $PSFBr_2$.[17] The latter are liquids, b. p. 35·5° and 125·3°; $PSFBr_2$ is stated to be less reactive than PSF_2Br, and to show unusual resistance to hydrolysis by alkalis.

In spite of the frequent occurrence of "mixed" halides among other non-metals, attempts to prepare boron chlorofluorides by conventional reactions have been entirely unsuccessful.[18] Reaction of boron trichloride with antimony trifluoride (in presence of antimony pentachloride) at different temperatures, and of boron trifluoride with calcium fluoride at 200°, both result in formation of boron trifluoride containing none of the "mixed" analogues. Rearrangement does not occur when mixtures of boron trichloride and trifluoride are heated or passed through an electric discharge.

The formation of stable additive compounds between boron halides (particularly the fluoride) and electron-donating molecules is well known, and further attention has been given to such compounds. Methylboron difluoride and dimethylboron fluoride both react with trimethylamine, forming the compounds $BMeF_2,NMe_3$ and BMe_2F,NMe_3, respectively.[19] The boron–nitrogen bond in the former compound is markedly less stable than that in BF_3,NMe_3, but the mono- and the di-methyl derivative do not differ appreciably in this respect. Free energies of dissociation have been estimated for these compounds in order to provide a quantitative measure of the effect of substitution on the strength of the boron–nitrogen bond.

Hexamethylenetetramine, $(CH_2)_6N_4$, would be expected to form an additive compound with four molecular proportions of boron trifluoride; such a compound has now been prepared by adding the trifluoride to a solution of the tetramine in liquid sulphur dioxide.[20] As the temperature rises, this compound evolves boron trifluoride until the composition of the residue approximates to $(CH_2)_6N_4,BF_3$, but no discontinuities in gas evolution occur that would indicate the existence of stable compounds intermediate in composition between this and the original $(CH_2)_6N_4,4BF_3$.

Thionyl and sulphuryl chlorides do not react with boron trifluoride or

[16] J. A. Gierut, F. J. Sowa, and J. A. Nieuwland, *J. Amer. Chem. Soc.*, 1936, **58**, 897.

[17] H. S. Booth and C. A. Seabright, *ibid.*, 1943, **65**, 1834.

[18] H. S. Booth and S. G. Frary, *ibid.*, p. 1836.

[19] A. B. Burg and (Miss) A. A. Green, *ibid.*, p. 1838.

[20] A. B. Burg and LaV. L. Martin, *ibid.*, p. 1635.

trichloride; phosphoryl chloride does not react with boron trifluoride, but with the trichloride the compound $POCl_3,BCl_3$ is formed.[21]

A modification of H. Meerwein and W. Pannwitz's procedure for the preparation of the dihydrate of boron trifluoride [22] has recently been described.[23] A product melting sharply at 5·9—6·1° is prepared by passing 1 mol. of boron trifluoride into 2 mols. of water cooled in ice, or by absorbing an excess of the trifluoride in water and adding the requisite quantity of water to give the composition $BF_3,2H_2O$. Distillation of the dihydrate, even at pressures down to 1 mm., causes some decomposition, successive fractions showing a small variation in density. Distillation under 25 mm. yields a fraction of b. p. 85°, identified as dihydroxyfluoboric acid, $H_3BO_2F_2$, which can be redistilled without decomposition even at atmospheric pressure. A higher-boiling fraction from the dihydrate appears to be a mixture of several molecular species. In dioxan solution boron trifluoride dihydrate is considerably dissociated, as cryoscopic measurements show, and it is probable that the liquid material contains an equilibrium mixture of hydroxyfluoboric acids.

Since organosilicon compounds are almost exclusively prepared from silicon halides as starting materials, it is appropriate to consider them at this point. Recent work on the simpler derivatives includes the preparation of trimethylsilane from trichlorsilane and methylmagnesium bromide, and the direct conversion of the trimethylsilane into trimethylchlorosilane by treatment with chlorine at $-20°$.[24] Trimethylchlorosilane has also been prepared by reaction of methylmagnesium chloride with a mixture of methylchlorosilanes in ethereal solution.[25] The reawakening of interest in organosilicon compounds, due to their potential industrial value in the field of synthetic resins, lends importance to the chemistry of the simpler members of the group.

Other recent developments in organosilicon chemistry include the isolation of trimethylsilanol,[26] $SiMe_3{\cdot}OH$, a colourless liquid prepared by hydrolysis of the reaction product of methylmagnesium iodide and dimethylsilicone :

$$\{SiMe_2O\}_x + x\text{MgMeI} \longrightarrow x\text{SiMe}_3{\cdot}\text{OMgI}$$
$$\text{SiMe}_3{\cdot}\text{OMgI} + H_2O \longrightarrow \text{SiMe}_3{\cdot}\text{OH} + \text{MgIOH}$$

(cf. ref. 27). A second method is the ammonolysis of trimethylchlorosilane to hexamethyldisilamine, $SiMe_3{\cdot}NH{\cdot}SiMe_3$, and treatment of this product with dilute hydrochloric acid :

$$\text{SiMe}_3{\cdot}\text{NH}{\cdot}\text{SiMe}_3 + 2H_2O + HCl \longrightarrow 2\text{SiMe}_3{\cdot}\text{OH} + NH_4Cl$$

[21] A. B. Burg and M. K. Ross, *J. Amer. Chem. Soc.*, 1943, **65**, 1637.
[22] *J. pr. Chem.*, 1934, **141**, 123.
[23] J. S. McGrath, G. G. Stack, and P. A. McCusker, *J. Amer. Chem. Soc.*, 1944, **66**, 1263.
[24] A. G. Taylor and B. V. de G. Walden, *ibid.*, p. 842.
[25] W. F. Gilliam and R. O. Sauer, *ibid.*, p. 1793.
[26] R. O. Sauer, *ibid.*, p. 1707.
[27] F. S. Kipping and J. E. Hackford, *J.*, 1911, **99**, 138.

Trimethylsilanol is more readily dehydrated than higher silanols, prolonged refluxing at room temperature resulting in loss of water. Desiccants also promote dehydration. Hexamethyldisiloxane, Si_2OMe_6, has also been prepared by hydrolysis of trimethylchlorosilane. Methoxy- and ethoxytrimethylsilane have been prepared by the action of the corresponding alcohols on trimethylchlorosilane in toluene and xylene solution, respectively, in presence of pyridine. The *n*-butoxytrimethyl compound is obtained by direct interaction of trimethylchlorosilane and *n*-butanol. These trimethylsilyl ethers form azeotropic mixtures with the corresponding alcohols. The action of phosphoric oxide on hexamethyldisiloxane gives tris(trimethylsilyl) phosphate, $(SiMe_3)_3PO_4$.

Metallic Oxides.—In the past, incomplete understanding of the nature of metal–oxide systems has frequently led to the formulation of " lower oxides " of apparently anomalous composition. A comprehensive study of the oxides of tungsten [28] has now clarified a particularly difficult case of this kind. Mixtures of tungsten and its trioxide were prepared, covering in 19 steps the whole range of net compositions between $WO_{0\cdot10}$ and $WO_{2\cdot98}$; these mixtures were heated in argon at 800°, and the products examined by Debye–Scherrer *X*-radiograms and density and electrical conductivity measurements. The results show clearly the existence of four distinct phases in the composition range given; the α-phase, WO_3, is replaced at slightly lower oxygen contents ($WO_{2\cdot92}$ to $WO_{2\cdot88}$) by a closely-related but supposedly distinct β-phase of lower symmetry; the γ-phase, representing the intermediate oxide variously formulated by previous workers as W_2O_5 or W_4O_{11}, has a stability range between $WO_{2\cdot76}$ and $WO_{2\cdot65}$, the formula W_4O_{11} appearing to be correct; the WO_2 phase (δ) is stable in the range $WO_{2\cdot03}$—$WO_{2\cdot00}$. In the intermediate ranges ($WO_{2\cdot88}$—$WO_{2\cdot76}$ and $WO_{2\cdot65}$—$WO_{2\cdot03}$) two-phase systems occur. Similar systems are obtained by reduction of tungsten trioxide with hydrogen under suitably controlled conditions. Products apparently containing combined water, obtained by reduction of tungstic acid instead of the trioxide, are regarded as analogues of the tungsten bronzes, and formulated as WO_3,nH_2 ($n < 1$) (cf. tungsten bronzes, WO_3,nNa).[29] Supposed hydroxides, $W_4O_{10}(OH)_2$ and $W_{12}O_{32}(OH)_2$,[30] are probably of this tungsten-bronze type.

The formation of " tungsten-blue " has also been the subject of recent study.[31] Reduction of suspensions of tungsten trioxide by acid stannous chloride solution or by zinc and hydrochloric acid yields a blue product which is readily reoxidised to the trioxide by atmospheric oxygen; it gives substantially the same *X*-radiogram as tungsten trioxide, and corresponds with the α-phase referred to above. It is, in fact, tungsten trioxide with a small proportion of the oxygen atoms removed from the lattice. Reduction of M-ammonium tungstate solution with acid stannous chloride gave a very

[28] O. Glemser and H. Sauer, *Z. anorg. Chem.*, 1943, **252**, 144.
[29] G. Hägg, *Z. physikal. Chem.*, 1935, *B*, **29**, 192.
[30] F. Ebert and H. Flasch, *Z. anorg. Chem.*, 1934, **217**, 95; 1936, **226**, 65.
[31] O. Glemser and H. Sauer, *ibid.*, 1943, **252**, 160.

similar product, again readily reoxidised, giving an *X*-radiogram identical with that of tungstic acid. However, if the ammonium tungstate is reduced by zinc and hydrochloric acid, a tungsten-blue stable to atmospheric oxygen is obtained; this is found to correspond with the hydrogen analogue of tungsten bronzes, referred to above. This substance is also obtained, in admixture with some free metal, by the action of sulphuric acid on tungsten under various conditions.

The existence and stability of rhodium oxides have recently been studied by methods involving " degradation isotherms," or curves showing the variation of equilibrium oxygen pressure during isothermal removal of oxygen from the oxide phase.[32] Previous work [33] had indicated the existence of the oxides Rh_2O_3, RhO, and Rh_2O, together with a hydrated dioxide, RhO_2,nH_2O; the existence of Rh_2O and RhO was inferred from the fact that phases corresponding in composition with these oxides gave oxygen dissociation pressures (p) such that $\log p$ varied linearly with $1/T$, the $\log p$–$1/T$ curves for each supposed oxide being distinct. The degradation isotherm of rhodium trioxide, Rh_2O_3, at 1050°, shows, however, that only one lower oxide, RhO, is formed as oxygen is removed from Rh_2O_3, the oxygen pressure of Rh–RhO at this temperature being 219 mm. Re-examination of the previous oxygen-pressure data at different temperatures shows that the supposed curves for RhO and Rh_2O cannot be sharply distinguished.

The oxygen dissociation pressure of rhodium trioxide is considerably reduced by admixture of magnesium, beryllium, calcium, or zinc oxide, owing to the formation of compounds (of the type MgO,Rh_2O_3) corresponding with the well-known group of spinels; the existence of these compounds as solid phases is shown by well-marked horizontal sections on the degradation isotherms obtained at 1100°. The magnesium and the zinc compound are capable of taking appreciable proportions of the oxides of these metals into solid solution. With oxides of other bivalent metals (cobalt, nickel, and copper) the systems become more complex owing to formation of compounds (such as $Cu_2Rh_2O_4$) other than those of the simple spinel type. Systems containing a high proportion of cupric oxide in admixture with rhodium trioxide give an oxygen pressure greater than that of cupric oxide alone, the reaction $2CuO + 2CuO,Rh_2O_3 \longrightarrow 2(Cu_2O,Rh_2O_3) + O_2$ occurring instead of $4CuO \longrightarrow 2Cu_2O + O_2$. Formation of mixed crystals by rhodium trioxide and aluminium, chromium, or manganese trioxide or ferric oxide causes interesting variations of oxygen pressure; cerium dioxide and silica have the apparently anomalous effect of increasing this pressure. This paper illustrates very well the large amount of useful information which can be derived from accurately executed studies based on phase-rule principles.

Phase-rule methods have also been applied to a study of the behaviour of ferric oxide towards added oxides of other elements at temperatures in the

[32] R. Schenck and F. Finkener, *Ber.*, 1942, **75**, 1962.

[33] L. Wöhler and W. Müller, *Z. anorg. Chem.*, 1925, **149**, 125; L. Wöhler and K. F. A. Ewald, *ibid.*, 1931, **201**, 145; L. Wöhler and N. Jochum, *Z. physikal. Chem.*, 1933, **167**, 169.

neighbourhood of 1300°.[34] The effect of adding the foreign oxide is inferred from the resulting change of the oxygen dissociation pressure due to the reaction $6Fe_2O_3 \longrightarrow 4Fe_3O_4 + O_2$. Silica and aluminium and beryllium oxides have little effect; chromic oxide and titanium dioxide are shown to yield solid solutions of high stability, ferric titanate being formed in the latter case. With manganese trioxide a spinel, MnO,Fe_2O_3, is obtained, ferric oxide behaving as a " foreign oxide " which promotes reduction of the manganese to the bivalent state.

The formation of so-called " ortho-salts " by addition of sodium oxide to a variety of supposedly " neutral " salts has already been described.[35] Some novel new compounds of similar type have recently been discussed.[36] The oxides of bivalent zinc, copper, nickel, and cobalt all give additive compounds with sodium oxide on sintering the components at 350—450°. The products of highest sodium oxide content are Na_6ZnO_4, Na_4CuO_3, Na_2NiO_2, and Na_4CoO_3, respectively. Evidence was obtained for the existence of other similar compounds containing smaller proportions of sodium oxide. The chemical individuality of the compounds formulated above has been fully established by *X*-ray powder diagrams. Structurally the compounds appear to tend towards a class of which the spinels are characteristic, in which closely-bound structural units do not occur in the lattice. They may possibly, however, show some transition towards structures with distinct anion groupings; their compositions, and the fact that no corresponding magnesium compound can be prepared, in spite of similar ionic dimensions of magnesium and the four metals concerned, suggest that bonds of other than polar type exist in the solid structures. Clearly the increasing range of compounds of this character provides scope for the application of crystal chemistry.

Binary Compounds of Metals (*other than Oxides*).—A nitride of nickel, Ni_3N, has been described;[37] it is prepared by heating nickel or its fluoride or bromide in ammonia at 445°. Although soluble in mineral acids, this nitride is not decomposed by sodium hydroxide solution; it appears to be an interstitial compound, the nickel atoms forming a hexagonal close-packed lattice into which the nitrogen atoms are packed.

The chemistry of metallic phosphides is now receiving increased attention. An electrolytic method which has been successful in other cases has been applied to the preparation of phosphides of copper;[38] two compounds, Cu_2P and Cu_3P, are distinguished, both being obtained (together with metallic copper) at the cathode on electrolysis of fused sodium metaphosphate containing dissolved cuprous chloride or cupric oxide. Both phosphides are oxidised in warm air, and Cu_2P readily evolves phosphorus on heating.

In the literature no less than five different phosphides of aluminium have

[34] N. G. Schmahl, *Z. Elektrochem.*, 1941, **47**, 835.
[35] E. Zintl and W. Morawietz, *Z. anorg. Chem.*, 1938, **236**, 372.
[36] G. Woltersdorf, *ibid.*, 1943, **252**, 126.
[37] R. Juza and W. Sachsze, *ibid.*, 1943, **251**, 201.
[38] M. Chêne, *Compt. rend.*, 1942, **214**, 977; **215**, 81.

been reported, and a careful study of the preparation and composition of aluminium phosphide [39] is therefore welcome. A phosphide was obtained most satisfactorily by placing an intimate mixture of aluminium powder and excess of red phosphorus in a Pyrex-glass reaction tube, displacing air in the tube by hydrogen, heating some phosphorus in the hydrogen stream to provide some phosphorus in the vapour phase, and igniting the mixture by strong local heating of part of the tube in contact with it. A vigorous reaction proceeded through the mixture. Unreacted phosphorus was afterwards removed from the product by sublimation. A special method of analysis was developed to permit determination of phosphorus combined as phosphide, free aluminium, total aluminium, and phosphoric oxide in a single sample of the substance. From the analytical results, and from the fact that only a single Debye–Scherrer *X*-radiogram could be obtained from preparations containing aluminium and phosphorus in different ratios, it is concluded that only one phosphide of aluminium, AlP, is obtained by the method of preparation described; this method yields a product containing about 94% of AlP. Various other methods of preparation tested failed to give phosphides of different composition. The phosphide AlP is a grey or yellowish-grey crystalline solid which in the absence of moisture or other reactants does not melt, sublime, or decompose at temperatures up to 1000°. It reacts readily with water, and still more readily with acids or alkalis, to give phosphine which is not spontaneously inflammable in air at ordinary temperatures. This investigation provides an interesting example of the application of a specially devised method of analysis in the solution of a difficult problem.

Metallic phosphides, together with the more numerous and better known sulphides, have also been considered in relation to systematic affinity theory,[40] and interesting generalisations relating to the number and type of sulphides and phosphides formed by different elements have resulted. These should provide a useful guide in further research in this field.

Basic Salts, etc.—Considerable research into the composition and nature of basic salts has been carried out recently, the wider availability of *X*-ray diffraction methods having provided the necessary means of establishing the individuality of solid phases. W. Feitknecht and his collaborators have continued their series of studies on basic salts of bivalent metals with four papers on zinc and cadmium hydroxyhalides, in which a careful systematic study is made of the conditions of preparation and the structures of basic salts falling into several classes.[41]

In work on the precipitation of phosphates, many of them basic, the technique of " acidimetric precipitation " has been introduced; [42] potassium

[39] W. E. White and A. H. Bushey, *J. Amer. Chem. Soc.*, 1944, **66**, 1666.

[40] W. Biltz, *Z. physikal. Chem.*, 1941, *A*, **189**, 10.

[41] W. Feitknecht and H. Weidmann, *Helv. Chim. Acta*, 1943, **26**, 1560, 1564; W. Feitknecht and H. Bucher, *ibid.*, pp. 2177, 2196.

[42] W. Rathje, *Ber.*, 1941, **74**, 342; F. Giesecke and W. Rathje, *ibid.*, p. 349; W. Rathje, *ibid.*, pp. 357, 546.

or sodium dihydrogen phosphate solution is added slowly to a boiling dilute solution of the nitrate or chloride of the metal, neutrality of the solution being maintained by simultaneous, controlled addition of sodium hydroxide solution. Although a number of metals give normal orthophosphates, $M_3^{II}(PO_4)_2$ (M^{II} = Mg, Ba, Cd, Mn, Fe^{II}, Co, Ni, Cu) or $M^{III}PO_4$ (M^{III} = Al, La, Ce, Bi), by this procedure, yet basic salts of the general formula $3M_3^{II}(PO_4)_2,M^{II}(OH)_2$ (M^{II} = Ca, Sr, Zn, Pb) are formed in other cases; the formal relation of these salts to apatite $[3Ca_3(PO_4)_2,CaF_2]$ is evident, and it is noteworthy that when $3Ca_3(PO_4)_2,Ca(OH)_2$ is treated with dilute sodium fluoride solution, partial exchange of hydroxyl and fluoride ions takes place.

A single basic magnesium nitrate, $Mg(NO_3)_2,4Mg(OH)_2$, is stated to be produced by addition of alkali carbonate or hydroxide solutions to 4—5M-solutions of magnesium nitrate; more dilute solutions of the magnesium salt afford the hydroxide, $Mg(OH)_2$, as the stable solid phase.[43]

An interesting anomaly is observed when phosphoric acid solution is titrated with calcium hydroxide; after rather more than one equivalent of the alkali has been added, further additions *lower* the pH of the solution. During prolonged shaking subsequently the pH rises slowly.[44] It has recently been pointed out [45] that when one equivalent of base has been added to the phosphoric acid, the solution contains $Ca^{\cdot\cdot}$, HPO_4'', and H_2PO_4' ions together with undissociated $CaHPO_4$. This may act as an acid during further addition of calcium hydroxide and give a precipitate (possibly $CaOH,CaPO_4$) containing calcium and phosphorus in a ratio of at least 2 : 1. Removal of $CaHPO_4$ by this precipitation lowers the value of HPO_4'' and consequently reduces the pH. If a suitable seed-crystal is present, the initial precipitate may decompose on keeping into $Ca_3(PO_4)_2$ or a related compound, the base again being liberated into the solution and causing the pH to rise.

A new ammine of basic cupric chromate, $2CuO,4NH_3,CrO_3,H_2O$, has recently been described.[46] Deposition of a crystalline tetramminocupric chromate, $Cu(NH_4)_4CrO_4$, from ammoniacal cupric chromate solution has been observed previously; the new compound is obtained in small yield from solutions deficient in chromium and containing a considerable excess of ammonia, and separates as deep blue-green crystals when the solution is kept at room temperature.

The nature of the pigment known as "zinc yellow" has long been a subject of controversy, some investigators regarding it as a basic zinc chromate of definite composition, and others as an adsorption complex. The recent preparation [47] of crystalline potassium, sodium, and ammonium salts of the type $M_2^IO,4ZnO,4CrO_3,3H_2O$ is therefore of interest; these salts are obtained by reaction of an aqueous suspension of zinc oxide with solutions of alkali (or ammonium) tetrachromate, $M_2^ICr_4O_{13}$. Well-

[43] (Mme.) L. Walter-Lévy, *Compt. rend.*, 1943, **216**, 846.
[44] G. A. Wendt and A. H. Clarke, *J. Amer. Chem. Soc.*, 1923, **45**, 881.
[45] I. Greenwald, *ibid.*, 1944, **66**, 1305. [46] W. H. Hartford, *ibid.*, p. 312.
[47] O. F. Tarr, M. Darrin, and L. G. Tubbs, *ibid.*, p. 929.

crystallised specimens of the basic salts were obtained in each case by slow neutralisation of a hot acid solution of the original product containing an excess of dichromate.

The existence of a normal chromate of indium appears doubtful in the light of recent work.[48] The yellow precipitate obtained by addition of potassium chromate to a solution of an indium salt, after filtration and thorough washing, contains only about one-tenth of the amount of chromium required by the formula $In_2(CrO_4)_3$. It appears likely that the precipitate formed is a basic salt; if the normal chromate exists at any stage of the precipitation, it is rapidly hydrolysed.

A basic nitrite of lanthanum containing La_2O_3 and N_2O_3 in the ratio of approximately 1 : 1 is obtained by making a faintly acid solution of lanthanum chloride 0·5—2M, with respect to sodium nitrite, and subsequently boiling it.[49]

Reference may be made here to the isolation of an interesting new series of hydroxy-salts, the *cadmates*, which can be crystallised from solution of cadmium hydroxide in concentrated aqueous alkalis.[50]. Typical members of this series are $Na_2[Cd(OH_4]$ and $Sr_2[Cd(OH)_6]$; a compound formulated as $Na_2[Cd(OH)_{3\cdot75}Br_{0\cdot25}]$ affords evidence that at least part of the hydroxyl may be replaced by a halogen. Attempts to prepare corresponding mercurates were unsuccessful.

Silicates, Aluminates, etc.—Several recent contributions to the study of silicates and compounds of similar type, which provide a wide and useful field of research, deserve brief mention. In a further paper of a series dealing with silicate chemistry, a detailed study of the synthesis of topaz, $Al_2SiO_4(F,OH,O)_2$, is described.[51] Topaz is obtained by heating a mixture of anhydrous aluminium fluoride and silica; the reaction proceeds rapidly between 750° and 950°, these temperature limits being sharply defined. Below 750° and above 950° a different solid product, resembling mullite in structure but containing 5—6% of fluorine, results. Formation of topaz is said to occur through intermediate formation of silicon tetrafluoride, the sequence of reactions being as follows : $4AlF_3 + 6H_2O \longrightarrow 2Al_2O_3 + 12HF$; $12HF + 3SiO_2 \longrightarrow 3SiF_4 + 6H_2O$; $2Al_2O_3 + SiF_4 + SiO_2 \longrightarrow 2Al_2SiO_4F_2$. Water adsorbed on the solid reactants is regarded as sufficient to permit this mechanism. Formation of the solid phase of mullite type apparently occurs by direct reaction between solids. In the course of this work a new oxyfluoride of aluminium, formulated as $Al_7O_{10}F$, was characterised; this is formed by the action of steam on anhydrous aluminium fluoride at temperatures less than 600°.

In a further study of the same series [52] some reactions of anhydrous pyrophyllite, $Al_2(Si_4O_{10})O$, have been examined and compared with corres-

[48] M. F. Stubbs, *J. Amer. Chem. Soc.*, 1923, **45**, 498.
[49] G. R. Sherwood, *ibid.*, p. 1228.
[50] R. Scholder and E. Staufenbiel, *Z. anorg. Chem.*, 1941, **247**, 259.
[51] R. Schober and E. Thilo, *Ber.*, 1940, **73**, 1219.
[52] E. Thilo and U. Schwarz, *ibid.*, 1941, **74**, 196.

ponding reactions of talc, the magnesium analogue. Pyrophyllite itself does not decompose below 1150°, but in presence of magnesium oxide decomposition occurs at 900°, magnesium spinel ($MgAl_2O_4$) and silica (or magnesium silicates) being produced; it is to be noted that 900° is also the temperature at which decomposition of talc begins. A similar reaction (affording a copper spinel, $CuAl_2O_4$, and silica) occurs readily with cupric oxide at 950°. With magnesium chloride pyrophyllite reacts on heating to give cordierite ($2MgO,2Al_2O_3,5SiO_2$), but cobaltous chloride gives cobalt spinel ($CoAl_2O_4$), silica, and hydrogen chloride, with an unidentified blue solid. Thermal decomposition of pyrophyllite is concluded to occur by disruption of the layers of silicon–oxygen tetrahedra into simple fragments.

Phase-rule methods have been used in a study of relatively complex systems involving potassium and calcium carbonates, silica, and various derived silicates.[53]

Continued interest in hydrothermal reactions is shown by recent work on the decomposition of tricalcium aluminate ($3CaO,Al_2O_3$) by steam at 350°;[54] the products are $4CaO,3Al_2O_3,3H_2O$ and calcium hydroxide, the former being dehydrated to $12CaO,7Al_2O_3$ and alumina in air at 650—750°.

The formation of alkali metasilicates from silica and sodium oxide, sodium or potassium carbonate, or sodium bicarbonate has been studied recently.[55] The reaction of anhydrous sodium carbonate and amorphous silica in equimolecular proportion is substantially complete in 80 minutes at 875°. At lower temperatures reaction proceeds readily to a point such that about half the carbon dioxide is expelled from the reaction mixture, but further reaction is slow; *X*-radiograms give no evidence of formation of an intermediate product at this stage.

Complex Compounds.—Although the field is wide and difficult to review, interesting advances in the chemistry of complex compounds have been recorded recently, complexes of the platinum metals having received particular attention; discussion of the considerable bulk of Russian work on this subject has been deferred to a later Report.

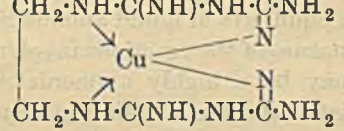
Special interest attaches to a wide range of complexes derived from cupric azide, $Cu(N_3)_2$; the preparation and properties of the crystalline azide itself, and of two basic azides, $Cu(OH)N_3$ and $Cu(N_3)_2,2Cu(OH)_2$, have been described in considerable detail.[56, 57] The first group of complexes [58] comprises compounds closely analogous to the halides of complex cupric cations containing co-ordinated ammonia or amines; $[Cu(NH_3)_4](N_3)_2$ and $\left[Cu\begin{pmatrix}CH_2\cdot NH_2\\CH_2\cdot NH_2\end{pmatrix}_2\right](N_3)_2$ are typical of this group. Another group [59] includes numerous compounds formulated as non-electrolytes, such as

[53] C. Kröger, K. W. Illner, and W. Graeser, *Z. anorg. Chem.*, 1943, **251**, 270.
[54] H. Johnson and T. Thorvaldson, *Canadian J. Res.* 1943, **21**, *B*, 236.
[55] G. F. Hüttig and K. Dimoff, *Ber.*, 1942, **75**, 1573.
[56] M. Straumanis and A. Cīrulis, *Z. anorg. Chem.*, 1943, **251**, 315.
[57] A. Cīrulis and M. Straumanis, *ibid.*, p. 332.
[58] M. Straumanis and A. Cīrulis, *ibid.*, p. 335.
[59] A. Cīrulis and M. Straumanis, *ibid.*, p. 341.

$[Cu(NH_3)_2(N_3)_2]$ and $[Cu(C_5H_5N)_2(N_3)_2]$. Other non-electrolytes [60] derived from a wide variety of simple and complex organic amines include compounds of the general types $[Cu(amine)_2(N_3)_2]$, $[Cu(amine)(N_3)_2]$, and $[Cu(N_3)_2(amine)Cu(N_3)_2]$; the apparent tercovalency of the copper atom in many of these compounds merits further study. A totally different class of complex is furnished by the "azidocuprates" in which copper occurs in the anion; [61] these are of the type $M_4^I[Cu(N_3)_6]$, $M_2^I[Cu(N_3)_4]$, $M^I[Cu(N_3)_3]$, and $M^I[(N_3)_2CuN_3Cu(N_3)_2]$, the cations in the compounds described being alkali or alkaline-earth metals or organic bases. Tercovalent copper appears to occur in many of these complexes also. In the later papers of the series the range of azidocuprates with organic cations is extended,[62] and more detailed attention is given to the azidocuprates of potassium, rubidium, and strontium.[63] Although the potassium compound, $K[Cu(N_3)_3],H_2O$, and the strontium compound, $Sr[Cu(N_3)_4],3H_2O$, appear to possess mononuclear anions, the rubidium and the cæsium salt are of the type $M^I[Cu_2(N_3)_5]$, evidently involving a binuclear complex. Attempts to prepare beryllium and magnesium compounds were unsuccessful, and although evidence for the existence of a calcium compound was obtained, this was not isolated. The B sub-group metals of Groups I and II do not give azidocuprates. It is noteworthy that in lithium azidocuprate, six azide groups are co-ordinated per copper atom, and three molecules of water of hydration may occur; in the rubidium and the cæsium salt the co-ordination has fallen to five azide groups per pair of copper atoms, and hydrates are not obtained. The effect of cation size on co-ordination in the anion is thus well illustrated, and structural studies of these compounds by *X*-ray methods will be awaited with interest. Interesting transitions also occur in the stability and solubility relations in this range of compounds, and in the facility with which they crystallise from solutions. As expected, many of the complexes of cupric azide detonate on percussion.

Other interesting examples of copper complexes include a group of complex cuprous thiosulphates [64] of which the simpler types are $M^ICu_2S_2O_3,nH_2O$ and $M_4^ICu_2S_2O_3,nH_2O$. Copper also gives an interesting stable complex ion with ethylenebisdiguanide,[65] affording a number of salts of the type $[RH_2]X_2,nH_2O$, where the group R has the structure (I) and X is a univalent anion.

(I.)



[60] A. Cīrulis and M. Straumanis, *J. pr. Chem.*, 1943, **162**, 307.
[61] M. Straumanis and A. Cīrulis, *Z. anorg. Chem.*, 1943, **252**, 9.
[62] A. Cīrulis and M. Straumanis, *Ber.*, 1943, **76**, 825.
[63] M. Straumanis and A. Cīrulis, *Z. anorg. Chem.*, 1943, **252**, 121.
[64] G. Spacu and J. G. Murgulescu, *Kolloid-Z.*, 1940, **91**, 294.
[65] P. Rây and S. P. Ghosh, *J. Indian Chem. Soc.*, 1943, **20**, 291.

Related nickel and cobalt compounds also exist. Complexes of ethylenebisdiguanide with tervalent silver are known.[66]

The co-ordination number of bivalent lead has been discussed recently.[67] Although a compound with thiourea, $Pb(NO_3)_2,6CS(NH_2)_2$, has been prepared, indicating a possible co-ordination number of six, this compound is extensively dissociated in boiling solution and may be an additive molecular compound.

Continuing a series of studies on the chromammines, C. L. Rollinson and J. C. Bailar, jun., have investigated the preparation of diacidobisethylenediamine salts by thermal decomposition of related trisethylenediamine (luteo) complexes.[68] The salt $[Cr\ en_3](SCN)_3,H_2O$ loses its water of crystallisation and one-third of its ethylenediamine on heating at 130°,

$$trans\text{-}[Cr\ en_2(SCN)_2]SCN$$

being formed. The corresponding chloride, $[Cr\ en_3]Cl_3,3{\cdot}5H_2O$, decomposes in a similar manner at 160° giving *cis*-$[Cr\ en_2Cl_2]Cl$. In both cases the decomposition is catalysed to a marked extent by traces of the ammonium salt corresponding with the luteo-salt cation, the action of which appears to be specific; this peculiar catalytic effect, which merits further investigation, suggests that the reaction mechanism involves entry of negative ions from the catalyst molecules into the complex nucleus. The reactions described provide convenient and efficient methods for the preparation of the diacidobisethylenediamine salts.

The reactions of sexavalent chromium compounds with liquid ammonia have recently been studied systematically.[69] Addition of chromic anhydride or potassium chlorochromate to liquid ammonia at — 33° results in formation of a yellow-tan solid material of evidently complex composition; analysis shows that in this material about one in four of the originally sexavalent chromium atoms has been reduced to the tervalent state. The gaseous nitrogen recovered from the reaction represents about half that equivalent to the chromium reduced; the "missing" nitrogen can be recovered by heating the solid residue, and appears to be held chemically in some chromium complex. Treatment of ammonium dichromate with liquid ammonia causes reduction of about one-eighth of the chromium; ammonium chromate does not react. Ammonium nitrate, dissolved in the liquid ammonia, increases the extent to which the sexavalent chromium is reduced, but water has the opposite effect. Although these facts permit certain conclusions to be drawn concerning the equilibria in liquid ammonia solutions of chromium compounds, the precise nature of the reactions involved remains to be found. The yellow-tan solid may be a highly ammoniated chromic ammonium chromate or an ammoniated chromic amminochromate.

The chemistry of some new complex compounds of rhenium has been examined.[70] K_2ReCl_6 or K_3ReCl_6 in aqueous solution gives no complexes

[66] P. Rây and K. Chakravarty, *J. Indian Chem. Soc.*, 1944, **21**, 47.
[67] R. C. Haworth and F. G. Mann, *J.*, 1943, 661.
[68] *J. Amer. Chem. Soc.*, 1944, **66**, 641. [69] H. H. Sisler and F. E. Jirik, *ibid.*, p. 1344.
[70] V. V. Lebedinski and B. N. Ivanov-Emin, *J. Gen. Chem. Russ.*, 1943, **13**, 253.

with ammonia, pyridine, or thiourea, but with a large excess of ethylenediamine the yellow crystalline compound $[ReO_2\,en_2]Cl$ is obtained; other salts of the same complex cation can be obtained from this by double decomposition. An aqueous solution of $[ReO_2\,en_2]Cl$ undergoes marked changes of colour on addition of hydrochloric acid; at pH 2·8—3·2 the solution is red, and precipitates $[ReO(OH)\,en_2]Cl_2$ when alcohol is added; at still higher acid concentrations the colour changes through violet to deep blue (8N-acid), $[Re(OH)_2\,en_2]Cl_3$ being obtained from the blue solution.

Attempts have been made to prepare cyanato-complexes of tervalent cobalt.[71] It is found, however, that the action of potassium or silver cyanate on aquocobaltammine salts affords carbamato-complexes by a rapid exothermic reaction in which OCN′ ions add directly to the co-ordinated water molecules. The carbamato-cobaltic complex ions of which salts are described are $[Co(NH_3)_5CO_2{\cdot}NH_2]^{\cdot\cdot}$ and $[Co(NH_3)_4(H_2O)CO_2{\cdot}NH_2]^{\cdot\cdot}$.

Although potassium nickelocyanide, $K_2Ni(CN)_4$, is familiar and other related salts are known, a number of nickelocyanides of metals and complex cations, described recently,[72] are new.

Further work on the chemistry of bivalent rhodium complexes forms a useful recent contribution to the study of the simpler types of complex compound. A number of compounds containing dialkylarsines as co-ordinated addenda have been prepared;[73] they are of the types

$$[RhX_2(AsMe_2R)_4],\quad \left[Cl(AsMe_2R)_3Rh\begin{matrix}\nearrow Cl\\ \searrow Cl\end{matrix}Rh(AsMe_2R)_3Cl\right],$$

$[Rh(AsMe_2R)_6][RhI_5AsMe_2R]$, and $[Rh(AsMe_2R)_6][RhI_4(AsMe_2R)_4]$, where X is a halogen and R an aryl group. These compounds are obtained, generally, by reduction of related complexes of tervalent rhodium with hypophosphorous acid in presence of the free alkylarsine. A range of bivalent rhodium halide complexes with pyridine has also been described.[74] Treatment of the compound $[Rh\,py_6]Br_3$ in solution at 100° with hypophosphorous acid affords $[Rh\,py_6]Br_2$, isolated as yellow crystals. This compound, by appropriate reactions, gives $[Rh\,py_5Br]Br$, $[Rh\,py_4Br_2]$,

$$\left[py_3BrRh\begin{matrix}\nearrow Br\\ \searrow Br\end{matrix}RhBr\,py_3\right],\quad (py\,H)_2\left[py_2Br_2Rh\begin{matrix}\nearrow Br\\ \searrow Br\end{matrix}RhBr_2py_2\right],$$

$$(py\,H)_4\left[py\,Br_3Rh\begin{matrix}\nearrow Br\\ \searrow Br\end{matrix}RhBr_3py\right],\text{ and }(py\,H)_6\left[Br_4Rh\begin{matrix}\nearrow Br\\ \searrow Br\end{matrix}RhBr_4\right].$$

Similar compounds containing halogens other than bromine have been prepared in some cases. The existence of bivalent rhodium in both cationic

[71] M. Linhard and H. Flygare, *Z. anorg. Chem.*, 1943, **251**, 25.

[72] F. Feigl, V. Demant, and O. E. do Oliveira, *Anal. Asoc. Quím. Brasil*, 1944, **3**, 72.

[73] F. P. Dwyer and R. S. Nyholm, *J. Proc. Roy. Soc. N.S.W.*, 1943, **76**, 133.

[74] *Idem, ibid.*, p. 275.

and anionic complexes is confirmed by a polarographic study of solutions of tervalent rhodium salts.[75]

In view of the known existence of a compound, $K_4Ni(CN)_4$, in which nickel appears to show zero valency, the isolation of an analogous compound of palladium, $K_4Pd(CN)_4$,[76] is of interest. This is obtained by the action of metallic potassium on a solution of $K_2Pd(CN)_4$ in liquid ammonia; a compound $K_2Pd(CN)_3$, corresponding with a known complex cyanide of univalent nickel, apparently does not occur as an intermediate product.

A. J. E. W.

2. Gallium.

Although many minerals have been investigated, particularly in Russia, no important new sources of gallium have been discovered during the past few years. Small quantities have been found in topazes and beryls, in the mineral deposits of Kazakstan, and in a number of coals.[1] Other sources include the waste materials of the zinc industry, zinc blendes, in Switzerland as well as Russia, and the waste products of the non-ferrous metallurgy in the Urals.[2] Outside Russia, small amounts of gallium have been discovered in ores in America, in the rocks of Virginia,[3] in Greenland, in rocks of the Skaergaard intrusion,[4] and in Japan in certain hot springs.[5]

Much work has been carried out recently on the compounds of gallium. The element has a uniform valency of three and gives no series of compounds of any other valency, although a suboxide GaO has been described, as well as a dichloride $GaCl_2$, formed by heating the trichloride with the metal.

The system gallium oxide–water resembles the alumina–water system closely. At 300—1400° the stable form is β-Ga_2O_3, which is insoluble in water and mineral acids. At 100—300° GaO(OH) is formed by heating Ga_2O_3 with water; it is soluble in mineral acids. In an atmosphere containing water vapour under pressure, the metastable gallium trihydroxide is sometimes formed at 167—170°. Once formed, both these hydroxides are fairly stable, but at high temperatures they are both converted into β-oxide. The α-oxide is difficult to separate, but can be obtained by precipitating a dilute boiling solution of gallium trichloride with sodium carbonate, and drying the precipitate at 425°. The dehydration curves of gallium hydroxide give some indication of the formation of yet another hydroxy-

[75] J. B. Willis, *J. Amer. Chem. Soc.*, 1944, **66**, 1067.

[76] J. J. Burbage and W. C. Fernelius, *ibid.*, 1943, **65**, 1484.

[1] S. A. Borovik, *Compt. rend. Acad. Sci. U.R.S.S.*, 1941, **31**, 24; S. K. Kalinin, *Bull. Acad. Sci. U.R.S.S.*, Sér. phys., 1941, 253; V. M. Kostrikin and B. N. Ivanov-Emin, *J. Appl. Chem. U.S.S.R.*, 1940, **13**, 1498.

[2] V. I. Babikova and Z. A. Gornova, *Tsvet. Met.*, 1940, No. 5—6, 121; F. I. Abramov and A. K. Rusanov, *Soviet Geol.*, 1938, **8**, No. 5, 64; G. Beck, *Mitt. Naturforsch. Ges. Berne Sitzber.*, 1937, **5**—**6** (1938); V. S. Syrokomskii and A. K. Sharova, *Tsvet. Met.*, 1938, No. 11, 23.

[3] A. Matthews and H. Ussery, *Proc. Virginia Acad. Sci.*, 1940—41.

[4] L. R. Wagner and R. L. Mitchell, *Min. Mag.*, 1943, **26**, 283.

[5] Kazuo Kuroda, *Bull. Chem. Soc., Japan*, 1940, **15**, 234.

compound, $Ga_2O(OH)_4$.[6] The gelatinous precipitate obtained by adding alkali to a solution of a gallium salt is hydrous gallium oxide, which on standing in contact with ammonium hydroxide becomes the monohydrate Ga_2O_3,H_2O.[7]

The normal chloride of gallium, $GaCl_3$, closely resembles aluminium chloride, as would be expected from the positions of gallium and aluminium in the Periodic Table. It is readily prepared in quantitative yield by heating gallium metal at 200° in an atmosphere of hydrogen chloride. The product may be sublimed and kept in an anhydrous state in sealed tubes if the air is replaced by nitrogen.[8] Up to 200° it exists in the dimeric form Ga_2Cl_6.

The dichloride, $GaCl_2$, was prepared [9] by heating the trichloride in a tube at 175° with excess of gallium, and purified by sublimation in a vacuum. It forms colourless crystals, m. p. 170·5°, which deliquesce to a thick, colourless solution when exposed to air. In water, it forms a chocolate-coloured precipitate which reacts with water slowly, evolving hydrogen. Heated to above 200°, it disproportionates into gallium and the trichloride. Vapour-density determinations in the range 400—470° indicate that the vapour contains some $GaCl_2$ molecules, in which gallium must show the anomalous valency of 2. In the experiments no indication of a monochloride of gallium was obtained.

Gallium trichloride dissolves in many organic solvents to give yellowish solutions unless the solvents are especially purified, the solution then being colourless. From such solutions the additive compounds $GaCl_3$,PhCN, m. p. 125°, $GaCl_3$,*p*-$C_6H_4Me{\cdot}NO_2$, m. p. 95°, and $GaCl_3$,BzCl, m. p. 46°, have been separated.[10] It also reacts with dimethylzinc to give an 85% yield of trimethylgallium.[11] $2GaCl_3 + 3ZnMe_2 \longrightarrow 2GaMe_3 + 3ZnCl_2$. A better method, however, is to heat gallium metal with dimethylmercury, whereby a nearly quantitative yield of the dimethyl compound is obtained : [12] $2Ga + 3HgMe_2 \longrightarrow 2GaMe_3 + 3Hg$.

With ether, trimethylgallium forms the compound $GaMe_3,Et_2O$, which in turn dissolves in ammonia to give the ammoniate $GaMe_3,NH_3$ on evaporation. The latter, a white solid, m. p. 31°, reacts with potassium hydroxide according to the equation $GaMe_3,NH_3 + KOH \longrightarrow GaMe_2OK + CH_4 + NH_3$. Trimethylgallium ammoniate reacts with sodium in liquid ammonia according to the equations : [13]

$$2[GaMe_3,NH_3] + Na \longrightarrow (GaMe_3)_2NH_2Na + \tfrac{1}{2}H_2 + NH_3$$
$$2[GaMe_3,NH_3] + 2Na \longrightarrow (GaMe_3)_2Na_2 + 2NH_3$$

[6] A. W. Laubengayer and H. R. Engle, *J. Amer. Chem. Soc.*, 1939, **61**, 1210.

[7] H. B. Weiser and W. O. Milligan, *Chem. Reviews*, 1939, **25**, 1.

[8] W. C. Johnson and C. A. Haskew, "Inorganic Syntheses," New York, McGraw-Hill Book Co., 1939, p. 26.

[9] A. Laubengayer and F. Schirmer, *J. Amer. Chem. Soc.*, 1940, **62**, 1578.

[10] H. Ulich and G. Heipe, *Z. physikal. Chem.*, 1941, *B*, **49**, 284.

[11] C. Kraus and F. Toonder, *Proc. Nat. Acad. Sci.*, 1933, **19**, 292.

[12] E. Wiberg, Th. Johannsen, and O. Stecker, *Z. anorg. Chem.*, 1943, **251**, 114.

[13] C. Kraus and F. Toonder, *J. Amer. Chem. Soc.*, 1933, **55**, 3547.

Similarly with lithium in liquid ethylamine $GaMe_3,NH_2Et + Li \longrightarrow GaMe_3,NHEtLi + \frac{1}{2}H_2$. If dimethylgallium chloride is reduced with sodium in liquid ammonia, dimethylgallium is obtained. This also forms a solid ammine $GaMe_2,NH_3$, which decomposes slowly, according to the equation $GaMe_2,NH_3 \longrightarrow GaMe_2{\cdot}NH_2 + \frac{1}{2}H_2$. With sodium the probable reaction is $GaMe_2,NH_3 + Na \longrightarrow GaMe_2,NaNH_2 + \frac{1}{2}H_2$.

Trimethylgallium is also decomposed by iodine or hydrogen iodide at room temperature :

$$GaMe_3 + 3I_2 \longrightarrow GaI_3 + 3MeI$$
$$GaMe_3 + 3HI \longrightarrow GaI_3 + 3MeH$$

The reaction with tertiary amines produces crystalline, difficultly volatile, white addition compounds of the composition GaM_3,NR_3. The trimethylamine derivative is a white solid, m. p. 96·2°, which dissociates partly into its components on vapourising; $GaMe_3,NEt_3$ melts at 97° and boils with decomposition at 167°.

Both triethyl- and triphenyl-gallium have been prepared by the method, already stated for trimethylgallium, of heating the metal with the appropriate alkyl- or aryl-mercury, triethylgallium by A. Laubengayer and W. Gilliam,[14] and triphenylgallium by H. Gilman and R. G. Jones.[15] The phenyl compound is recrystallised from chloroform; short exposure to dry air has little effect, but it is decomposed by moisture.

The bromides and iodides of gallium are formed by direct union of the elements. Fluorides are also known. The trihydrate $GaF_3,3H_2O$ is obtained by dissolving the metal or the oxide in hydrofluoric acid, and the anhydrous salt may be obtained by thermal decomposition of ammonium gallium fluoride $(NH_4)_3GaF_6$ in fluorine. If solutions of metal fluorides in hydrofluoric acid are mixed with gallium fluorides, the following mixed fluorides result:[16] Li_3GaF_6, Na_3GaF_6, $K_2(GaF_5,H_2O)$, $Rb(GaF_4,2H_2O)$, $Cs(GaF_4,2H_2O)$, $(NH_4)_3GaF_6$, $3SrF_6,GaF_3,3H_2O$, $Ba_3(GaF_6)_2,H_2O$, $Ag_3(GaF_6),10H_2O$, $Tl(GaF_5,H_2O)$, and six of the composition $[M(H_2O)_6][GaF_5,H_2O]$ where M = Cu, Zn, Cd, Mn, Co, or Ni.

The fluorides of gallium also form ammoniates.[17] Gallium fluoride has a low activity towards ammonia, and hence it is necessary first to prepare the hydrate $GaF_3,3H_2O$ by dissolving the nitrate or oxide in 40% hydrofluoric acid and crystallising the salt obtained. The hydrate is extracted 8—10 times with liquid ammonia, and is then treated with gaseous ammonia at room temperature. Finely powdered $GaF_3,3NH_3$ is obtained. On being heated, it shows a marked dissociation pressure at 100° and a pressure of one atmosphere at 163°. The occurrence of a diammoniate $GaF_3,2NH_3$ is indicated, but because of thermal decomposition it is not definitely established.

The gallium salts of many of the oxyhalogen acids are known. The

[14] *J. Amer. Chem. Soc.*, 1941, **63**, 477. [15] *Ibid.*, 1940, **62**, 980.
[16] E. Einicke, *Die Chemie*, 1942, **55**, 40.
[17] W. Klemm and H. Kilian, *Z. anorg. Chem.*, 1939, **241**, 93.

chlorate monohydrate, $Ga(ClO_3)_3,H_2O$, and the bromate may be obtained from a solution of gallium sulphate by addition of a solution of barium chlorate or bromate, and the iodate monohydrate is formed equally simply by addition of hydriodic acid to a solution of gallium in nitric acid.

Of the gallium salts of oxyhalogen acids, the perchlorate has been studied in greater detail than the others. Gallium dissolves readily in concentrated perchloric acid, to give a clear solution from which gallium perchlorate hexahydrate may be separated.[18] A second hydrate $Ga(ClO_4)_3,9\frac{1}{2}H_2O$ was also described, but D. Lloyd and W. Pugh [19] state that this is the nonahydrate, which they prepared from gallium nitrate and a slight excess of perchloric acid. If this is dehydrated with phosphoric oxide or sulphuric acid, the hexahydrate is again obtained. It decomposes on heating according to the equation $4[Ga(ClO_4)_3,9H_2O] \longrightarrow 2Ga_2O_3 + 36H_2O + 6Cl_2 + 21O_2$. Evidence has also been obtained by Lloyd and Pugh for the formation of a basic perchlorate $3Ga_2O_3,Ga(ClO_4)_3$.

Gallium perchlorate readily forms a complex with urea when alcoholic solutions of the nonahydrate and urea are mixed. The complex, $[Ga(CON_2H_4)_6](ClO_4)_3$, m. p. 179°, is readily soluble in water, but decomposes to give gallium hydroxide. The crystals decompose with violence on heating. Attempts to prepare a complex with pyridine and with thiourea were unsuccessful.

Until recently, it was thought that all elements up to four places before a rare gas in the Periodic Table, together with boron, formed volatile hydrides. It now seems that this rule can be extended to five places before a rare gas to include hydrogen compounds of the third group of the Periodic System. So far, two hydrogen compounds of gallium have been prepared.[20] Trimethylgallium reacts with hydrogen in a glow discharge to give a colourless viscous liquid of formula $Ga_2H_2Me_4$, which decomposes above 130° according to the equation $3Ga_2H_2Me_4 \longrightarrow 4GaMe_3 + 2Ga + 3H_2$. At room temperature the compound reacts with triethylamine to give a volatile gallium hydride Ga_2H_6 and a triethylamine compound of trimethylgallium : $3Ga_2H_2Me_4 + 4NEt_3 \longrightarrow 4GaMe_3,NEt_3 + Ga_2H_6$.

Recently, a borohydride of gallium has been described.[21] The borohydrides known until then were $LiBH_4$, $CH_3{\cdot}BeBH_4$, $Be(BH_4)_2$ and $Al(BH_4)_3$. When, however, trimethylgallium is treated with an excess of diborane at room temperature, a small decrease in pressure occurs; a metallic film suddenly appears, accompanied by an increase in pressure and the formation of hydrogen. The film was identified as gallium, and for each mol. of trimethylgallium taken, approximately 3 mols. of methylated diborane, 1·5 mols. of hydrogen, and 1 mol. of gallium were obtained. The reaction therefore appears to be $GaMe_3 + 3B_2H_6 \longrightarrow Ga + 3CH_3{\cdot}B_2H_5 + \frac{3}{2}H_2$.

[18] L. Foster, *J. Amer. Chem. Soc.*, 1939, **61**, 3122.
[19] *J.*, 1943, 76.
[20] E. Wiberg and Th. Johannsen, *Die Chemie*, 1942, **55**, 38.
[21] H. Schlesinger, H. Brown, and G. Schaeffer, *J. Amer. Chem. Soc.*, 1943, **65**, 1786.

It seems probable that gallium borohydride is first formed, but this then undergoes rapid autocatalytic decomposition :

$$2GaMe_3 + 9B_2H_6 \longrightarrow 2Ga(BH_4)_3 + 6B_2H_5Me$$
$$2Ga(BH_4)_3 \longrightarrow 2Ga + 3B_2H_6 + 3H_2$$

Reaction between diborane and trimethylgallium at −45° results in formation of pure dimethylgallium borohydride, a volatile crystalline solid, m. p. 1·5°, which undergoes slow decomposition at room temperature : $2GaMe_3 + 3B_2H_6 \longrightarrow 2GaMe_2BH_4 + 2B_2H_5Me$.

Gallium forms salts with all strong acids. It dissolves readily in nitric acid, for example, to give a solution from which the octahydrate may be crystallised. A basic nitrate, $Ga(OH)_2NO_3,Ga(OH)_3,2H_2O$, is also known.[16]

Other compounds containing nitrogen and gallium have also been prepared. These are the nitrides Ga_3N_4 and GaN, which have been prepared by H. Hahn and R. Juga;[22] both are formed by heating the metal in a stream of nitrogen, and GaN may also be prepared by heating ammonium gallium fluoride $(NH_4)_3GaF_6$ to 600° in ammonia. The former is a bright grey solid which does not burn readily or completely to the oxide, and the latter is a bright grey or yellow solid which burns in oxygen according to the equation $4GaN + 3O_2 \longrightarrow 2Ga_2O_3 + 2N_2 - 415{\cdot}2$ kg.-cals.

With sulphuric acid, gallium forms a normal sulphate which is not deliquescent, and from which many alums have been prepared; *e.g.*, $R_2H_2SO_4,Ga_2(SO_4)_3,24H_2O$, where $R = NH_2{\cdot}OH$, *iso*-$C_5H_{11}{\cdot}NH_2$, or *iso*-$C_4H_9{\cdot}NH_2$.[23] Pseudo-alums, $(NR'R_2)_2H_2SO_4,Ga_2(SO_4)_3,18H_2O$, have been prepared by the same workers, $(NR'R_2)$ representing either diethyl- or trimethyl-amine.

A double sulphate, $Ga_2(SO_4)_3,C_2H_4(NH_3)_2SO_4,12H_2O$, has been obtained by mixing saturated solutions of ethylenediamine sulphate and gallium sulphate; it is precipitated by addition of alcohol and ether. The corresponding compound has also been prepared from propylenediamine sulphate.[24]

A basic ammonium gallium sulphate, $(NH_4)_2SO_4,Ga_2(SO_4)_3,2Ga_2O_3,8H_2O$, is also known. It is obtained as a precipitate by keeping a solution of ammonium gallium sulphate at room temperature.[16]

Gallium phosphate is obtained anhydrous by heating an alkylgallium salt solution with phosphoric acid in a sealed tube at 200°, or as trihydrate, by dissolving freshly prepared gallium hydroxide in phosphoric acid. Two hypophosphites are known : $GaH_3(PO_2)_2$, obtained from gallium hydroxide and hypophosphorous acid, and $GaPO_2,H_2O$, obtained by treating a solution of gallium nitrate with a solution of sodium hypophosphite.

Gallium arsenate is prepared as the dihydrate by precipitation or by heating a gallium salt with arsenic acid in a Carius tube to 200°.

The gallium salts of a number of organic acids have been prepared.

[22] *Z. anorg. Chem.*, 1940, **244**, 111.
[23] P. Neogi and K. Mondal, *J. Indian Chem. Soc.*, 1942, **19**, 67.
[24] *Idem, ibid.*, p. 501.

A basic formate $Ga(CO_2H)_3,Ga_2O_3,5H_2O$ is obtained by dissolving gallium hydroxide in formic acid, and a basic acetate $Ga(OAc)_3,3Ga_2O_3,6H_2O$, tartrate $Ga_2(C_4H_4O_6)_3,4H_2O$, and oxalate $Ga_2(C_2O_4)_3,3Ga_2O_3,7H_2O$ are also known. Gallium "alizarate," prepared by the interaction of aqueous solutions of the potassium salt and gallium nitrate,[24] has the composition $\left[C_6H_4\langle\begin{matrix}CO\\CO\end{matrix}\rangle C_6H_2\langle\begin{matrix}O^-\\OH\end{matrix}\right]_3Ga$. It is insoluble in water, but soluble in alcohol and ammonia. It is a fast dye for cotton. If dissolved in ammonia, it forms a violet solution from which a dull red precipitate of calcium gallium alizarate $Ca_3Ga_2(C_{14}H_6O_4)_6$ may be obtained by addition of a solution of calcium chloride. Other salts described by the same workers include the maleate, obtained by dissolving gallium hydroxide in maleic acid solution, and the salts of *d*- and *l*-camphorsulphonic acid, obtained similarly.

Quite recently, attempts have been described to prepare a carbonyl of gallium.[25] A compound of the type $[Co(CO)_4]_3Ga$ may have been prepared, but so far only qualitative evidence for its formation has been obtained.

A number of solid systems involving gallium have been studied, and several intermetallic compounds described. The system Ag–Ga–Zn, studied by *X*-ray methods and by microscopic analysis,[26] shows that at high temperatures a compound Ag_3Ga is formed. With gold, gallium forms two compounds,[27] AuGa, which separates from melts of equiatomic composition at a maximum temperature of 468°, and $AuGa_2$, which crystallises above a maximum m. p. of 492°. Titanium and gallium form the compound $TiGa_3$,[28] and magnesium forms the compounds Mg_5Ga_2 and Mg_2Ga. *X*-Ray diagrams indicate that in the Ga–Mg system other phases exist with greater gallium content.

Hitherto, metallic gallium has found but limited use. It is claimed to improve the strength of magnesium–manganese alloys,[29] and to increase markedly the ductility and strength of magnesium.[30] Gallium was tried as a substitute for silver in dental fillings,[31] but incorporation of the metal in the solid Ag–Sn alloy gave alloys which were too soft for dental work.

The chlorides of gallium may be used as catalysts. Gallium trichloride is effective as a catalyst for the reaction between ethylene and benzene;[32] the optimum temperature is 50—60° and good yields of ethylbenzene are obtained. Gallium dichloride, too, has been found to be more active than aluminium chloride in the synthesis of ketones and hydrocarbons.[33]

S. R. R.

[25] W. Hieber, H. Behrens, and U. Teller, *Z. anorg. Chem.*, 1942, **249**, 43.
[26] K. Moeller, *Z. Metallk.*, 1939, **31**, 19.
[27] F. Weibke and E. Hesse, *Z. anorg. Chem.*, 1939, **240**, 289.
[28] F. Laves and H. Wallbaum, *Naturwiss.*, 1939, **27**, 674.
[29] U.S.P. 2,270,193.
[30] J. McDonald, *Trans. Amer. Inst. Min. Met. Eng.*, Inst. Metals Div., Tech. Pub. No. 1247 (1940).
[31] F. Weibke and E. Hesse, *Z. Elektrochem.*, 1940, **46**, 219.
[32] H. Ulich, A. Keutmann, and A. Geierhaas, *ibid.*, 1943, **49**, 292.
[33] H. Ulich, *Die Chemie*, 1942, **55**, 377.

3. Germanium.

Besides the main sources already well known, a number of new sources of germanium have been discovered in the past few years, although in no case was the percentage of germanium in an ore large. Most of the new sources were in Russia, where a large number of ores have been investigated. Germanium was found in small quantities in topazes and beryls,[1] in the mineral deposits of Kazakstan,[2] and in various other Russian rocks,[3] but by far the most abundant supply is in coals,[4] coal ash,[5] and in various flue dusts from either coals or cement.[6] Outside Russia, new deposits have been found in hot springs in Japan,[7] and in the flue dusts from Australian coals.[8]

A new extraction of germanium from germanite has been described,[9] which depends on the following facts : (i) Its dioxide is very slightly soluble in nitric acid. (ii) It is very soluble in ammonium oxalate, forming a complex. (iii) The ammonium germano-oxalate is not decomposed by hydrogen sulphide, a fact which allows a rapid and excellent separation of metals forming insoluble sulphides, but (iv) is decomposed to germanic oxide on heating with concentrated sulphuric acid. The finely ground mineral is attacked by concentrated nitric acid. The solid residue is treated with water to dissolve out any salts formed and leave germanic oxide, sulphur, lead sulphate, and part of the arsenic, molybdenum, tungsten, and iron.

The insoluble part is boiled for 24 hours with its own weight of a mixture of equal quantities of oxalic acid and ammonium oxalate, dissolved in the minimum quantity of water. The solution of germanic oxide is practically complete, forming ammonium germano-oxalate.

The oxalic acid liquor is treated with hydrogen sulphide, and after separation of the precipitated sulphides, is treated with ammonium sulphide. The remaining metals are precipitated in this way as the sulphides, except germanium which remains in solution as the complex, together with a little molybdenum and tungsten, the precipitation of which is never complete. The mixture is then heated to fuming with sulphuric acid and a little nitric acid. The precipitation of germanic oxide is practically complete. The sulphuric acid which contains the last impurities is decanted, and the precipitate is washed with water made ammoniacal, dried, and calcined. The ammonia removes the last traces of molybdenum and tungsten. The germanic oxide thus obtained is spectroscopically pure.

The determination of germanium has also received attention during the

[1] S. A. Borovik, *Compt. rend. Acad. Sci. U.R.S.S.*, 1941, **31**, 24.

[2] S. W. Kalinin, *Bull. Acad. Sci. U.R.S.S.*, Sér. phys., 1941, 253.

[3] S. A. Borovik, N. M. Prokopenka, and I. L. Pokrovskaya, *Compt. rend. Acad. Sci. U.R.S.S.*, 1939, **25**, 618.

[4] A. I. Egorov and S. W. Kalinin, *ibid.*, 1940, **26**, 925; V. A. Zilbermints, A. K. Rusanov, and V. M. Kostrikin, *Chem. Zentr.*, 1938, I, 1709.

[5] V. M. Kostrikin, *J. Appl. Chem.* (*U.S.S.R.*), 1939, **12**, 1449.

[6] W. Guertler, *Metall. Erz.*, 1940, **37**, 30, 46.

[7] Kazuo Kuroda, *Bull. Chem. Soc. Japan*, 1939, **14**, 303.

[8] W. T. Cooke, *Trans. Roy. Soc. S. Australia*, 1938, **62**, 318.

[9] A. Tchakirian, *Ann. Chim.*, 1939, **12**, 415.

past five years.[10] In two of the methods described, it is precipitated as germanomolybdic acid by ammonium molybdate and a solution of 8-hydroxyquinoline in acetic acid. The precipitate may be filtered off, and the germanium determined gravimetrically, or the complex broken down by boiling, and the germanium determined volumetrically. In the third method, the germanium is determined colorimetrically by means of ammonium molybdate–ferrous sulphate reagent. In all these determinations the initial germanium-containing material must first be broken down, usually by treatment with hydrogen fluoride and sulphuric acid, or, if chlorides are present, by fusion with sodium peroxide.

Another method for the quantitative determination of germanium makes use of a modified Marsh apparatus.[11] The germanium is extracted from the mineral by treatment with nitric, hydrofluoric, and sulphuric acids, and determined either by precipitation and gravimetric determination as germanic oxide, or by conversion into monogermane which is decomposed by heating to 360° in a tube, a ring of germanium being formed. The method is particularly recommended by its originators for small quantities (less than 1 mg.) of germanium. L. M. Dennis and R. P. Anderson,[12] too, claimed that it is possible to obtain a ring with 6×10^{-5} g. of germanic oxide by this method.

Germanium forms two oxides, germanous and germanic, each of which gives rise to a series of compounds. The sulphides, chlorides, and bromides are readily hydrolysable, but the iodides are less so. Germanium also forms penta- ($Ge_5O_{11}M_2$),[13] ortho- (GeO_4M_4),[14] meta- (GeO_3M_2),[15] and per-germanates ($Ge_2O_7M_2$ and GeO_5M_2).[16]

Germanium gives complex ions in which it has a valency of 4, and a co-ordination number of 6, *e.g.*, $M_2(GeF_6)$ [17] and $H_8Ge(W_2O_7)_6$.[18] Thiogermanates of the types $2GeS_2,3K_2S,9H_2O$ and $2GeS_2,3Na_2S,9H_2O$ are known, and germanic sulphide with piperazine gives the compound $GeS_2,C_4H_{10}N_2,SH_2$.[19]

Several nitrogen compounds have been prepared: Ge_3N_4,[20] $Ge(NH)_2$,[21]

[10] I. P. Alimarin and O. A. Alekseva, *J. Applied Chem.* (*U.S.S.R.*), 1939, **12**, 1900; W. Geilmann and E. Steuer, *Glastech. Ber.*, 1940, **18**, 89; A. G. Hybbinette and E. R. Sandell, *Ind. Eng. Chem. Anal.*, 1942, **14**, 715.

[11] W. C. Aitkenhead and A. R. Middleton, *ibid.*, 1938, **10**, 633.

[12] *J. Amer. Chem. Soc.*, 1914, **36**, 882.

[13] R. Schwarz, *Ber.*, 1929, **62**, 2477; R. Schwarz and E. Huf, *Z. anorg. Chem.*, 1931, **203**, 188.

[14] W. Pugh, *J.*, 1926, 2828; J. H. Müller, *J. Amer. Chem. Soc.*, 1922, **44**, 2493.

[15] C. A. Winkler, *J. pr. Chem.*, 1886, **34**, 213; W. A. Roth and O. Schwarz, *Ber.*, 1926, **59**, 338; W. Pugh, *J.*, 1929, 1992; J. H. Müller and C. E. Gulezian, *J. Amer. Chem. Soc.*, 1929, **51**, 2029.

[16] R. Schwarz and H. Giese, *Ber.*, 1930, **63**, 778.

[17] J. H. Müller, *Proc. Amer. Phil. Soc.*, 1926, **65**, Suppl. 5, 44.

[18] R. Schwarz and H. Giese, *Ber.*, 1930, **63**, 2430; A. Bruckl, *Monatsh.*, 1930, **56**, 179.

[19] L. Debucquet and L. Velluz, *Bull. Soc. chim.*, 1932, **51**, 1565.

[20] R. Schwarz and P. W. Schenk, *Ber.*, 1930, **63**, 296; W. C. Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 5160.

[21] R. Schwarz and P. W. Schenk, *loc. cit.*; W. Pugh and J. S. Thomas, *J.*, 1926, 1051.

Ge_2N_3H,[22] and GeNH.[23] Hydrides of the type Ge_nH_{2n+2} are known up to Ge_3H_8, as well as a solid hydride $(GeH)_n$. Known halogen derivatives are $GeHCl_3$, $GeHBr_3$, GeH_2Cl_2, GeH_3Cl, GeH_2Br_2, and GeH_3Br,[24] and the mixed halides $GeFCl_3$, GeF_2Cl_2, and GeF_3Cl [25] have recently been prepared. Also, two oxyhalides, Ge_2OCl_6 and $GeOCl_2$, are known.[26]

In spite of the relatively large amount of work on germanium, it was not until quite recently that germanous oxide and a number of the corresponding salts were prepared in a state of purity: Winkler and others could obtain only mixtures of germanous and germanic oxide by the action of sodium hydroxide on a mixture of germanium tetrachloride (30%) and germanochloroform (70%). The two methods described recently for the preparation of germanous oxide both involve the reduction of germanic salts, in one case by zinc and sulphuric acid and in the other by hypophosphorous acid.[9] In the first reduction, the solution must contain more than 25% of acid; the precipitate formed is dark brown, and after filtration and quick drying, is perfectly stable at room temperature. In the second preparation, germanic oxide is dissolved in sodium hydroxide, hydrochloric acid added until the solution is 3N, and after addition of hypophosphorous acid the solution is heated; reduction is considered complete when a sample added to sulphuric acid does not give a precipitate of germanic sulphate (about 2 hours).

By altering the conditions of the latter reduction it is possible to cause deposition of most of the germanium as a black precipitate, a small quantity being deposited on the walls of the flask as a reddish-grey mirror. Chemical analysis and *X*-ray study of the black precipitate and the mirror have not been able to prove whether they are lower oxides or mixtures of germanous oxide and metal. That lower oxides may be formed, is indicated by the fact that if the solution in which germanium has been reduced is treated with ammonia, hydrogen is evolved. Also, oxidation experiments with dilute nitric acid on the black powder and the mirror indicate the formulæ Ge_2O and Ge_3O.

Germanous hydroxide is orange-red; when heated in a current of carbon dioxide, or treated with concentrated sulphuric acid, it forms the dark brown oxide. It is soluble in hydrochloric and hydrobromic acids, but hydriodic acid converts it into the insoluble iodide. All salts, in slightly acid solution, give a bright orange precipitate of germanous sulphide with hydrogen sulphide. The sulphide is soluble in concentrated hydrochloric acid, and in ammonium sulphide to form the thiogermanate. All oxidising agents convert it into germanic oxide.

The hydroxide $Ge(OH)_2$ may be considered as being germanoformic acid

[22] R. Schwarz and P. W. Schenk, *loc. cit.*

[23] W. C. Johnson, G. Morey, and A. Knott, *J. Amer. Chem. Soc.*, 1932, **54**, 4278.

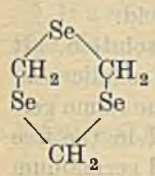
[24] C. A. Winkler, *J. pr. Chem.*, 1886, **34**, 222; F. M. Brewer and L. M. Dennis, *J. Physical Chem.*, 1927, **31**, 1527; L. M. Dennis and P. R. Judy, *J. Amer. Chem. Soc.*, 1929, **51**, 2321.

[25] H. Booth and W. Morris, *ibid.*, 1936, **58**, 90.

[26] R. Schwarz, P. W. Schenk, and H. Giese, *Ber.*, 1931, **64**, 362; R. Schwarz and F. Heinrich, *Z. anorg. Chem.*, 1932, **209**, 273.

($H{\cdot}GeO_2H$); its solubility is 0·18%. Conductometric measurements[27] show that it is a weak acid; with sodium hydroxide it forms a red solution of sodium germanoformate, which is oxidised atmospherically to sodium germanate.

The selenides of germanium, GeSe and $GeSe_2$, have been used as a method of detecting germanium. Hydrogen selenide reacts with germanium in acid solution to give a characteristic yellow precipitate.[28] A better method of detection, however, is to introduce hydrogen selenide into an aqueous solution of formaldehyde, in which it dissolves to form a fairly stable derivative, probably as inset, which reacts with germanium to give a yellow precipitate. The sensitivity of the test is 1 in 5,000,000. The presence of silicic, hydrocyanic, hydrofluosilicic, and other acids does not affect the test. If arsenic, tin, selenium, or other elements are present, however, it is necessary to add two drops of potassium fluoride solution before adding the hydrogen selenide–formaldehyde reagent. In the presence of fluoride ion, the germanium is precipitated very slowly with this reagent; the other elements present, *e.g.*, arsenic and tin, are precipitated and are filtered off, and a little aluminium sulphate added. The aluminium ion removes all the fluoride ion as AlF_6^{---}, and the germanium is then precipitated as usual.



A better method for the preparation of germanous and germanic selenide is to fuse together the correct amount of germanium and selenium.[29] Both selenides are somewhat soluble in acids and bases, and are oxidised by nitric acid to germanic oxide and selenious acid.

The phosphide of germanium, GeP, is formed in a similar way by fusing finely powdered germanium with excess of phosphorus to 400° in an evacuated tube.[30] The reaction is incomplete. *X*-Ray analysis shows that the products contain germanium and phosphorus in the ratio of 1 : 1, and have a structure distinct from both free germanium and free phosphorus. This conclusion is confirmed by tension experiments on mixtures heated to definite temperatures.

A number of systems between germanium and other metals have recently been studied. In only four cases, however, *viz.*, those of arsenic, iron, nickel and magnesium, are there maxima in the melting-point curves, showing the formation of intermediate compounds. Thermal analysis of the germanium–arsenic system gives two weak maxima at 732° and 737°, corresponding to the formation of $GeAs_2$ and GeAs respectively.[31] There are two maxima also in the system germanium–iron, at 1180°, corresponding to Fe_2Ge, and at 866°, corresponding to $FeGe_2$, and in the nickel–germanium system, there is a maximum melting point at 1200°, corresponding probably

[27] A. Hantzsch, *Z. anorg. Chem.*, 1902, **30**, 289.
[28] V. I. Kuznetsov, *J. Gen. Chem.* (*U.S.S.R.*), 1939, **9**, 1049.
[29] B. N. Ivanov-Emin, *ibid.*, 1940, **10**, 1813.
[30] M. Zumbusch, M. Heimbrecht, and W. Biltz, *Z. anorg. Chem.*, 1939, **242**, 237.
[31] H. Stohr and W. Klemm, *ibid.*, 1940, **244**, 205.

to Ni_2Ge.[32] Magnesium and germanium form a compound Mg_2Ge, m. p. $1115° \pm 5°$.[33]

Germanous hydroxide does not dissolve in tartaric acid, but a solution of the tartrate may be obtained by treating a solution of germanous chloride in dilute hydrochloric acid with sodium tartrate; from the resulting clear solution it is impossible to precipitate germanium with ammonia or with hydrogen sulphide except on long standing. These facts are taken to indicate the presence in the solution of a complex germanotartrate, in which 1 atom of bivalent germanium corresponds to 2 molecules of tartaric acid.

Germanic oxide is somewhat soluble in hot oxalic acid solution. It forms a complex in which the ratio of germanium atoms to oxalic acid molecules is 1 : 3, and to which the formula $[Ge(C_2O_4)_3]H_2$ and the name germanioxalic acid were given.[9] The acid could not be isolated in the free state, but that there is combination between the oxalic acid and germanium is shown by the fact that, if the solution is titrated with sodium hydroxide, neutralisation occurs when approximately three-quarters of the oxalic acid is accounted for. Similarly, if a mixture of potassium iodide and iodate is added, the quantity of the iodine liberated is always less than that which would be expected for the oxalic acid : even after 24 hours, the reaction is not complete.

Germanic oxide is much more soluble in ammonium oxalate than in oxalic acid. The complex is quite stable, for it is not decomposed by hydrogen sulphide, and sulphuric acid decomposes it to germanic oxide only on heating (this has been used in the separation of germanium from ores).

If a solution of germanioxalic acid is mixed with a concentrated solution of quinine oxalate, a salt $\{[Ge(C_2O_4)_3]C_{20}H_{24}O_2N_2\ H_2\}$ is precipitated immediately. The strychnine salt $\{[Ge(C_2O_4)_3]2C_{21}H_{22}O_2N_2\ H_2\}$ is formed in hot solution and precipitated on cooling. Both these salts are white powders and are very hygroscopic. Their analyses confirm the formula $[Ge(C_2O_4)_3]H_2$ for germanioxalic acid.

The solubility of germanic oxide in water is 6—8 g./l. If, however, the oxide is boiled with a solution of mannitol, a stable solution containing 10 g./l. is quickly obtained. This has been utilised in the determination of germanium by acidimetric and iodometric methods.

The end-point to phenolphthalein when a solution of germanic oxide is titrated with sodium hydroxide shows that 5 molecules of oxide are equivalent to 2 of the alkali. This coincides with the formula $(Ge_5O_{11})H_2$, which is analogous to metastannic acid $(Sn_5O_{11})H_2$. The existence of this acid is shown to be very probable by the work of A. Hantzsch,[34] who described the sodium salt, and by R. Schwarz,[35] who isolated the acid, obtained by dehydrating the hydrolysis product of germanium tetrachloride with sulphuric acid.

[32] K. Ruttewit and G. Masing, *Z. Metallk.*, 1940, **32**, 52.
[33] W. Klemm and W. Westlinning, *Z. anorg. Chem.*, 1941, **245**, 365.
[34] *Ibid.*, 1902, **30**, 316. [35] *Ber.*, 1929, **62**, 2477.

If mannitol, glycerol, glucose, or any other polyhydric organic compound is added to the above, it acts as a strong acid. A complex organogermanic compound is formed, so that on titration one atom of germanium corresponds to one molecule of sodium hydroxide. Addition of mannitol to pentagermanic acid causes a lowering of its association so that again a molecule of sodium hydroxide corresponds to an atom of germanium. Hence a formula such as $[Ge_2O_5(M)_n]H_2$ is possible, where M is mannitol and n a number at least equal to two.

If a mixture of potassium iodide and iodate is added to a solution of the mannitogermanic acid, an atom of iodine is liberated per atom of germanium present.[36] The reaction is probably

$$3[Ge_2O_5(M)_n]H_2 + KIO_3 + 5KI \longrightarrow 3[Ge_2O_5(M)_n]K_2 + 3H_2O + 3I_2$$

This fact allows germanic acid to be determined in the presence of strong acids. Excess of a mixture of potassium iodide and iodate is added to the solution, and the liberated iodine titrated to the end-point; the germanic acid is so weak as to have little effect on the titration, but on subsequent addition of mannitol and titration of the iodine further liberated, the latter is equivalent to the quantity of germanic acid present.

Mannitodigermanic acid is a strong acid. Its solution has pH 4·0 and liberates carbon dioxide from carbonates on boiling. It is a complex, for it gives no precipitate of germanic hydroxide with ammonia. If magnesium chloride is added to a solution of germanic oxide, a quantitative precipitation of magnesium germanate occurs, but in the presence of mannitol, the precipitation is not complete.

Besides mannitol, germanic acid forms complex salts with strong electrolytes, such as alkali sulphates, chlorides, or nitrates; the resulting solution is a strong acid. With concentrated solutions of calcium chloride or magnesium chloride, two molecules of the salt are combined per atom of germanium, indicating the existence of complex orthochlorogermanates, (I) and (II),

$$\left[\begin{matrix} & O_3 \\ Ge & \\ & Cl_n \end{matrix}\right]\begin{matrix} H_2 \\ \\ Ca_{n/2} \end{matrix} \qquad \left[\begin{matrix} & O_3 \\ Ge & \\ & Cl_n \end{matrix}\right]\begin{matrix} H_2 \\ \\ Mg_{n/2} \end{matrix} \qquad \left[\begin{matrix} & O_3 \\ Ge & \\ & A_n \end{matrix}\right]\begin{matrix} H_2 \\ \\ C_n \end{matrix}$$

(I.) (II.) (III.)

where n is undetermined.

In the case of the nitrates and chlorides of calcium and magnesium, the amount of sodium hydroxide required to neutralise the complex acid corresponds approximately to the complex (III), where A is the anion and C the cation of the strong electrolyte added. The temperature affects the formation of these complexes, since the amount of sodium hydroxide required on heating is greater than that in the cold. This leads us to suppose that these

[36] A. Tchakirian, *Compt. rend.*, 1928, **187**, 229; *Bull. Soc. chim.*, 1932, **51**, 846.

complexes are not formed instantaneously, and the molecule may undergo hydration to give salts different from the original, *e.g.*

$$2\left[\begin{matrix}\mathrm{Ge}\end{matrix}\begin{matrix}\mathrm{O_3}\\ \mathrm{A}_n\end{matrix}\right]\begin{matrix}\mathrm{H_2}\\ \mathrm{C}_n\end{matrix} + \mathrm{H_2O} \longrightarrow \left[\begin{matrix}\mathrm{Ge_2}\end{matrix}\begin{matrix}\mathrm{O_7}\\ \mathrm{A}_n\end{matrix}\right]\begin{matrix}\mathrm{H_6}\\ \mathrm{C}_n\end{matrix}$$

Germanous chloride, dissolved in hydrochloric acid with cæsium chloride, gives a white microcrystalline precipitate, $GeCsCl_3$. A similar compound is obtained with rubidium chloride, but not with the chlorides of lithium, sodium, potassium, calcium, strontium, and barium. T. Karantassis and L. Capatos have also prepared the corresponding bromides and iodides.[37]

The compound between germanous chloride and cæsium chloride may be either $GeCl_2$,CsCl or $CsGeCl_3$. The latter is regarded as the more likely, for the compound is not decomposed by a current of hydrogen chloride passed over it at the temperature of fusion, a fact which can hardly be reconciled with the formula $GeCl_2$,CsCl.

If a solution of germanous chloride in 7N-hydrochloric acid is electrolysed in a U-tube, at the end of 15 minutes a reddish precipitate of germanous hydroxide has been formed in the anode compartment, but there is no germanium in the cathode compartment. The explanation is that the ion $[Ge^{++}Cl_3]^-$ proceeds to the anode where it is neutralised and then hydrolysed to germanous hydroxide. Thus it may well be that in the cæsium compound, the formula is $CsGeCl_3$.

Several attempts were made to prepare alkyl- and aryl-substituted derivatives of germanium tetrachloride but in no case was the yield good. G. T. Morgan and H. D. K. Drew [38] treated germanium tetrachloride with phenylmagnesium bromide, R. Schwarz and M. Lewinsohn [39] tetraphenylgermanium with germanium tetrachloride, and E. A. Flood [40] germanous iodide with ethyl iodide; and W. R. Orndorff, D. L. Tabern, and L. M. Dennis [41] attempted the preparation of phenylgermanium trichloride by heating diphenylmercury with germanium tetrachloride in a sealed tube.

The latest method, which gives very good yields, is to heat cæsium germanium trichloride with an alkyl or aryl halide (preferably the iodide) in a sealed tube.[42] The reaction $RX + CsGeCl_3 \longrightarrow RGeCl_3 + CsX$ takes place. Hydrolysis of the resulting compounds gives rise to substituted germanoformic acid; *e.g.*, with methylgermanium trichloride, germanoacetic acid is formed: $CH_3{\cdot}GeCl_3 + 2H_2O \longrightarrow CH_3{\cdot}GeO_2H + 3HCl$. This reaction is reversible, for if the acid is treated with sufficiently concentrated hydrochloric acid, methylgermanium trichloride is regenerated.

Ethyl- and phenyl-germanium trichlorides have been prepared by this method, as well as the digermanium compound from methylene iodide,

[37] *Compt. rend.* 1934, **199**, 64; 1935, **201**, 74.
[38] *J.*, 1925, **127**, 1760. [39] *Ber.*, 1931, **64**, 2352.
[40] *J. Amer. Chem. Soc.*, 1933, **55**, 4935.
[41] *Ibid.*, 1927, **49**, 2512.
[42] A. Tchakirian, *Compt. rend.*, 1931, **192**, 233.

$CH_2(GeCl_3)_2$. If this compound is then hydrolysed by water, the germanium analogue of malonic acid is obtained as a white powder :

$$CH_2(GeCl_3)_2 + 4H_2O \longrightarrow CH_2(GeO_2H)_2 + 6HCl.$$

Tetra-substituted organo-germanium compounds of the type GeR_4 are known. The tetraphenyl derivative has been prepared by D. E. Worrall,[43] from phenylmagnesium bromide and germanium tetrachloride in toluene from which all the ether used to prepare the Grignard compound has been distilled, and also by refluxing germanium tetrachloride in toluene with sodium and bromobenzene. Schwarz and Lewinsohn prepared the compound phenylethyl*iso*propylgermanium bromide which is optically active.[39] There exist also organo-germanium compounds where the germanium appears to have valencies of three, two, and one.

Germanium appears as yet to have little commercial use. It is claimed [44] that germanium forms alloys with magnesium which are suitable for castings, and that reflectors of high efficiency may be made by evaporating germanium electrically in a highly evacuated chamber, and causing it to deposit on glass. The germanium is then backed by aluminium.[45]

The effect of the addition of germanic oxide to glass has been studied.[46] A partial or total replacement of silica in glass by germanic oxide causes an increase in the refractive index and in the dispersion. At the same time, however, it causes a lowering of the chemical stability of the glass.

S. R. R.

S. R. Robinson.
A. J. E. Welch.

[43] *J. Amer. Chem. Soc.*, 1940, **62**, 3267.
[44] U.S.P. 2,278,726.
[45] B.P. 508,205.
[46] K. Krakau, *Optiko-Mekhan. Prom.*, 1939, **9**, No. 4, 15.

ORGANIC CHEMISTRY.

1. Introduction.

This section contains for the first time a report on recent research on amino-acids, the account being limited almost entirely to natural α-amino-carboxylic acids and their near derivatives. In addition to improved preparations by well-established methods, attention is drawn to novel applications of the malonic ester synthesis to methionine, tryptophan, etc., to Hofmann and Curtius degradations of appropriate derivatives of cyanoacetic and malonic acid, and to the facile reduction of many arylhydrazones obtained by coupling diazotates with substituted malonic and similar esters. Recent analytical reactions of organic chemical interest are summarised and a brief account is given of the various applications of chromatography to amino-acids. The reaction between amino-acids and formaldehyde, the transamination reaction and the formation and behaviour of dehydration products of amino-acids and their near derivatives have been discussed as subjects of current interest. A notable feature of the material summarised under the last topic is the unexpected variety of the products, which include simple and poly-peptides, diketopiperazines, azlactones, and representatives of other heterocyclic series; still more versatile are the dehydropeptides and derivatives thereof obtained by extensions of the classical Ploechl–Erlenmeyer condensation of aldehydes with glycine derivatives.

Keten acetals are now readily available as a result of the investigations of S. M. McElvain and his collaborators, who have introduced two general methods of preparation: (i) by the elimination, with potassium *tert.*-butoxide, of hydrogen bromide from an α-bromo-acetal, and (ii) by the removal of the elements of alkyl hypobromite from an α-bromo ortho-ester by treatment with sodium. The ethylenic linkage is highly reactive in the majority of keten acetals, and enters into addition reactions either in the normal manner (reaction I) or by a type of 1 : 4-addition, in which two moles of the acetal are involved (reaction II).

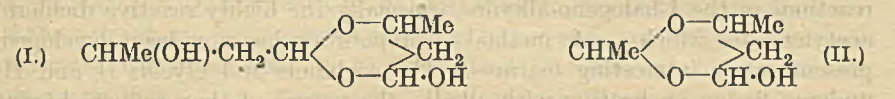
$$\text{I.}\quad CH_2{:}C(OEt)_2 + AB \longrightarrow A{\cdot}CH_2{\cdot}CB(OEt)_2$$

$$\text{II.}\quad 2CH_2{:}C(OEt)_2 + AB \longrightarrow A{\cdot}CH_2{\cdot}C(OEt)_2{\cdot}CH_2{\cdot}CB(OEt)_2$$

The adduct formed in either type of reaction is frequently unstable, and may decompose to give a variety of products, some of which may then react with a further quantity of keten acetal; in this way it is possible to account for the numerous by-products which are sometimes obtained. Reagents which readily form such adducts include alcohols, amines, phenols, acids, αβ-unsaturated carbonyl compounds, and diazonium salts. Keten diethyl-acetal is particularly sensitive towards traces of acids, which lead to the formation of chain polymers.

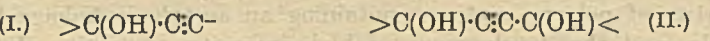
Considerable progress has recently been made in the elucidation of

problems concerning the structure of aldols and their dimeric forms. The balance of evidence appears to indicate that monomeric aldols are capable of existing in both the open-chain and the cyclic form, and it is now established that the dimers possess cyclic structures of the hydroxy-1 : 3-dioxan type, as exemplified by formula (I), now allocated to paraldol. Furthermore, it has been shown that monomeric aldols react with simple aldehydes



to give compounds of the same type. It follows that in the preparation of aldols the formation of an aldol–aldehyde adduct, such as (II), is unavoidable, and it is now known that crude aldols are largely composed of such material, which accounts for some of the divergent properties of " aldols " recorded in the literature. It has also been shown that in the presence of complex alkoxides, an aldol may react with an aldehyde in a different manner, by a crossed Cannizzaro reaction, to give a glycol ester. Following the successful outcome of the investigations on the dimers, it has been possible to make further progress in the allocation of structures to the higher condensation products of acetaldol, such as dialdan and tetra-aldan. This section of the Report contains also a brief account of some recent work on crotonaldehyde and its cyclic polymers.

In a summary of recent progress in the chemistry of acetylenic compounds a number of new and improved preparative methods for the hydrocarbons, including the vinylacetylenes, the carbinols (I), and the glycols (II), are



discussed. Distinct advantages accrue by operating in liquid ammonia, since sodamide, which is both readily formed and appreciably soluble in this medium, can be employed either for the dehydrohalogenation of halogenoolefins to acetylenes, or for the production of sodio-derivatives by replacement of the active hydrogen atom of acetylenic hydrocarbons. The sodio-compounds undergo smooth substitution reactions in liquid ammonia with alkyl halides and sulphates, yielding mono- or di-substituted acetylenes, and carbinols (I) are formed by addition to most types of carbonyl compounds. The Grignard reagents derived from monosubstituted acetylenes, which react with alkyl sulphates or reactive alkyl halides, giving various types of hydrocarbon, can be employed advantageously to obtain carbinols, and the dimagnesium bromide formed from acetylene itself still provides the most convenient laboratory route to the acetylenic glycols. Other processes employing potassium hydroxide and either acetylene or calcium carbide have been devised for the large-scale production of both carbinols and glycols. The isomerisation of acetylenes into allenes and conjugated dienes, and the catalytic conversion of *cyclo*hexylacetylenes into alkylbenzenes are discussed. Other topics which have received attention include the chlorination of the hydrocarbons, carbinols, and glycols in reactive solvents such as

methyl alcohol, the polymerisation of acetylenes with sulphur dioxide to polysulphones, and the formation of α-naphthol derivatives by condensation of arylacetylenes with diphenylketen. With amines in the presence of metallic salts, acetylene gives rise either to aminoacetylenes [*e.g.*, $CH_3{\cdot}CH(NHPh){\cdot}C{:}CH$] or to quinaldines. A variety of methods is available for the substitutive halogenation of acetylenic hydrogen atoms and the reactions of the 1-halogeno-alkynes, especially the highly reactive dichloroacetylene, for which a safe method of preparation has now been developed, present many interesting features. The carbinols and glycols (I and II) undergo fission on heating with alkalis, the nature of the products having been shown to be considerably dependent on the particular substituent groups present and on the particular reagent employed. Initial substitution, followed by rearrangements to halogeno-allenes and -dienes, results from treatment of the carbinols with halogen acids together with cuprous salts. The complex changes which occur, leading amongst other products to dioxan and tetrahydrofuran derivatives, when the carbinols and glycols are caused to react with either methyl alcohol or acetic acid by means of the mercuric oxide–boron trifluoride catalyst, have been largely elucidated. The carbinols and glycols derived from αβ-unsaturated carbonyl compounds readily isomerise in the presence of acids to conjugated vinylacetylenic alcohols. The general character of this novel type of anionotropic rearrangement has been demonstrated and the facile transformation of the glycol from octatrienal into a product containing a conjugated system of six ethenoid linkages and one triple bond may be cited as an example of outstanding interest. As a result of these investigations an examination of the light absorption properties of polyene systems containing an acetylenic linkage has been possible, and the development of high intensity absorption due to the production of the vinylacetylene chromophore on isomerisation has been utilised in kinetic studies. Interaction of vinylacetylene with aldehydes and ketones gives carbinols (*e.g.*, III), the reactions of which have received much attention. All hydration processes apparently involve initial isomerisation to the diethenoid ketones (*e.g.*, IV), which can then undergo various addition

(III.) $CH_2{:}CH{\cdot}C{:}C{\cdot}C(OH)Me_2 \longrightarrow CH_2{:}CH{\cdot}CO{\cdot}CH{:}CMe_2$ (IV.)

reactions, giving tetrahydro-γ-pyrones by addition of water and cyclisation, and β-alkoxy-ketones by reaction with alcohols.

Within recent years the significance of reactions between atoms or free radicals and molecules has become more widely recognised. Such reactions, which involve the symmetrical fission of an electron pair, are discussed under the heading Homolytic Reactions. Reactions of diazo and related compounds, which involve free-radical mechanisms, have been further utilised for synthetic purposes. The production of free radicals by the electrolysis of Grignard reagents has also been investigated, and Kharasch and his collaborators have developed a new method for the generation of free radicals in solution by means of the catalytic action of certain metallic halides on Grignard reagents. The capacity of acyl peroxides to yield free radicals on

thermal decomposition has led to the extensive use of such peroxides for the initiation of chain reactions in solution involving free radicals and atoms. Reactions of acyl peroxides have also been used in a number of investigations for the purpose of obtaining a clearer understanding of the chemical properties of free radicals of various types. Considerable advances have been made in our knowledge of addition polymerisation, which confirm the free-radical theory, since it has now been established that fragments of the catalyst and of the solvent are incorporated in the polymer and compounds other than peroxides, which can give rise to free radicals, are also capable of initiating polymerisation.

Growing recognition of the biological importance of nucleic acids has stimulated chemical investigation of these substances; the main outlines of the structures of the component nucleotides and nucleosides are now clear. The latter appear to be N_3-ribo- or -2-deoxyribo-furanosidopyrimidines and N_9-ribo- or -2-deoxyribo-furanosidopurines; in the nucleotides the phosphoryl group probably esterifies the hydroxyl group at C_3' of the carbohydrate residue. Synthetic studies have not yet resulted in complete synthesis of any natural nucleoside or nucleotide, but the close nucleoside analogues 3-*d*-ribopyranosidouracil and 9-*d*-ribopyranosidoadenine have been obtained by methods which should be capable of extension to the production of the naturally occurring furanosides, and several partial syntheses of natural nucleotides have been effected by phosphorylation of the corresponding nucleosides.

The chemistry of the heterocyclic compounds is represented in the Report by a discussion of the most recent work on the biotin substances, and by a review of progress made in the structural investigation of several groups of alkaloids. Inability to reconcile with his own observations the results obtained by V. du Vigneaud and his school in their study of biotin, led F. Kögl to conclude that the materials handled by the two groups of workers were not identical. This conclusion was confirmed by a direct comparison of the active substances isolated from egg-yolk and from liver. A stepwise degradation of α-biotin from egg-yolk afforded finally a sulphohexoic acid, the constitution of which was determined, and on the basis of this and earlier work, a structure has been advanced for α-biotin. The structure of β-biotin, obtained from liver or milk, has been confirmed by a complete synthesis of the substance. Two outstanding achievements in the chemistry of the cinchona alkaloids are reported: the first a total synthesis of quinine, and the second a complete elucidation of the stereochemical orientation of these alkaloids. Alstonine and a new *Rauwolfia* alkaloid, rauwolscine, have been shown to contain the β-carboline nucleus, and gelsemine also is shown to belong to the indole group. Hygroline, a new pyrrolidine base from coca, is closely related to hygrine. The structure previously assigned to retronecine has been fully confirmed. Some progress has been recorded in the difficult investigation of the aconite and veratrine groups. The close structural relationship between the aconite and *Delphinium* alkaloids has been conclusively demonstrated by the isolation of identical alkamines from

alkaloids of the two genera, and evidence has been obtained that the alkaloids of this group are related to the diterpenes. Further confirmation of the steroidal nature of solanidine is afforded by its transformation into four stereoisomeric solanidanols, closely analogous to the cholestanols; the veratrine bases rubijervine and *iso*rubijervine also appear to possess a regular steroid skeleton similar to that proposed for the *Solanum* group, but it is difficult to assign a like structure to the more highly oxygenated veratrine alkaloids on account of their different behaviour on degradation.

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2. Amino-Acids.

Introduction.

This Report, necessarily arbitrary in extent as it is the first on its topic in this Section, is confined to advances in the organic chemistry of those amino-acids which are constituents of proteins and a few nearly related acids, and of simple derivatives thereof. No attempt has been made to review aspects of biochemical or physicochemical interest or to summarise the growing literature on *p*-aminobenzoic and similar acids.[1] The Report covers the past 2 years with some earlier work to afford cohesion.

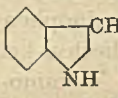
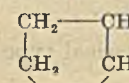
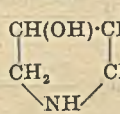
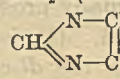
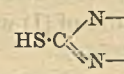
It is stated [2] that there are 18 amino-acids of general occurrence; glycine, leucine, tyrosine, serine, glutamic acid, aspartic acid, phenylalanine, alanine, lysine, arginine, histidine, valine, proline, tryptophan, hydroxyproline, *iso*leucine, methionine, threonine. To these must be added 7 acids of narrow distribution (thyroxine, di-iodotyrosine, dibromotyrosine, norleucine, cystine, cysteine, and hydroxyglutamic acid) and 5 of very limited occurrence but which may be present in proteins (thiolhistidine, dihydroxyphenylalanine, citrulline, canavanine and djenkolic acid), to which must probably be added lanthionine. There are finally about 20 acids which have not been completely substantiated, with rather vaguely defined amino-acids such as that believed to be present in liver antianæmia factor.[3]

[1] Reviews, etc.: C. Schmidt, "Chemistry of the Amino-acids and Proteins," 1938, with Addendum, 1943; M. Sahyun, "Outline of the Amino-acids and Proteins," 1944; E. J. Cohn and J. T. Edsall, "Proteins, Amino-acids and Peptides as Ions and Dipolar Ions," 1943; R. J. Block and D. Bolling, "Determination of Amino-acids," 1941; "Amino-acid Composition of Proteins and Natural Foods," 1944; "Amino-acid Chemistry," H. E. and L. E. Glynn, *Pharm. J.*, 1944, **153**, 22. See also volumes of *Ann. Rev. Biochem.* "Imidazole amino-acids," S. W. Fox, *Chem. Rev.*, 1943, **32**, 47.

[2] H. B. Vickery, *Annals N.Y. Acad. Sci.*, 1941, **41**, 87.

[3] J. Erdos, *Science*, 1942, **96**, 141.

The structures of the important members are summarised for convenience below,[4] with reference to R in the general formula $CHR(NH_2)\cdot CO_2H$:

		Nature of R.	R = .
NEUTRAL	ALIPHATIC	Straight chain	H, glycine. Me, alanine. *n*-Pr, norvaline. *n*-Bu, norleucine.
		Sym. chain	$CHMe_2$, valine. $CHMe_2\cdot CH_2$, leucine.
		Asym. chain	CHMeEt, *iso*leucine.
		Hydroxylated chain	$CH_2\cdot OH$, serine. $CHMe\cdot OH$, threonine. $CMe_2\cdot OH$, hydroxyvaline.
		Thiol chain	$CH_2\cdot SH$, cysteine (disulphide = cystine). $CH_2(SH)\cdot CH_2$, homocysteine.
		Thioether	$Me\cdot S\cdot CH_2\cdot CH_2$, methionine. $\cdot CH_2\cdot S\cdot CH_2\cdot S\cdot CH_2$ djenkolic acid.
	CYCLIC	Aryl	CH_2Ph, phenylalanine. *p*-$OH\cdot C_6H_4\cdot CH_2$, tyrosine, (dibromotyrosine, di-iodotyrosine). 2 : 4-$C_6H_4(OH)_2\cdot CH_2$, dihydroxyphenylalanine.
		Heterocyclic	 , tryptophan.
			 (proline).
			 (hydroxyproline).
	ACID	Straight chain	$CO_2H\cdot CH_2$, aspartic acid. $CO_2H\cdot CH_2\cdot CH_2$, glutamic acid. $CO_2H\cdot CH_2\cdot CH(OH)$, hydroxyglutamic acid.
	BASIC	Straight chain	$NH_2\cdot[CH_2]_3$, ornithine. $NH_2\cdot[CH_2]_4$, lysine. $NH_2\cdot O\cdot CH_2\cdot CH_2$, canaline.
		Guanidino-, ureo-, and hydroxylated chain	$NH_2\cdot C(:NH)\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2$, arginine. $NH_2\cdot CH_2\cdot CH(OH)\cdot CH_2\cdot CH_2$, hydroxylysine. $NH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2$, citrulline. $NH_2\cdot C(:NH)\cdot NH\cdot O\cdot CH_2\cdot CH_2$, canavanine.
		Iminazole	 , histidine.
		Thiol	 , thiolhistidine.

Synthesis of Amino-acids.

Of the classical methods, amination of α-halogenated acids, the Strecker cyanohydrin synthesis, and modifications of the malonic ester synthesis have been extensively employed, and a miscellany of new methods, some of fairly general application, are to be noted. Many reactions, discussed in a later section, such as those of the Ploechl–Erlenmeyer type (condensation

[4] This chart is constructed on the plan of that of G. Toennies, *Science*, 1943, **97**, 492, who includes much additional information.

of aromatic aldehydes with α-acylamino-acids to give 4-arylideneazlactones), amount virtually to syntheses of further amino-acids.

Optimum conditions for the amination of α-halogenated acids have been studied.[5] Leucine, *iso*leucine,[6] and valine [7] are thus obtainable in 30—40% yield from α-bromo-*iso*hexoic, -β-methylvaleric, and -*iso*valeric acid respectively; *dl*-phenylalanine arises in rather better yield even from crude α-bromo-β-phenylpropionic acid,[8] and a convenient synthesis of serine in 30—40% overall yield, utilising reactions which had all previously been used in the amino-acid field, involves a similar amination :[9]

$$CH_2{:}CH{\cdot}CO_2Me \xrightarrow{Hg(OAc)_2/MeOH} CH_2(OMe){\cdot}CH(HgOAc){\cdot}CO_2Me \xrightarrow{Br_2\text{–}KBr} CH_2(OMe){\cdot}CHBr{\cdot}CO_2Me \xrightarrow[\text{amination}]{\text{HBr hydrolysis}} \text{serine}$$

The Strecker synthesis, rather unsatisfactory with simple aldehydes, affords *ca.* 35% yield of pure *dl*-α-aminophenylacetic acid from phenylacetaldehyde,[10] and when applied to diethyl ketone [11] and acetophenone [12] it provides α-aminodiethylacetic and α-amino-α-phenylpropionic acids in *ca.* 40% yield.

An ingenious application of the classical type of malonic ester synthesis

$$CH_2Cl{\cdot}CH_2Cl \longrightarrow CH_2(S{\cdot}CH_2Ph){\cdot}CH_2Cl \xrightarrow[\text{malonic ester}]{\text{sodiophthalimido-}}$$

$$CH_2(S{\cdot}CH_2Ph){\cdot}CH_2{\cdot}C(CO_2Et)_2 \xrightarrow{Na/NH_3 + MeI} {}^*CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$$

(the phthalimido group $N<\begin{matrix}CO\\CO\end{matrix}>C_6H_4$ on the C bearing CO_2Et; SMe and NH_2 on the starred chain)

permitted the synthesis of methionine containing C* = ^{13}C, and ^{34}S with the object of utilising the doubly isotopic amino-acid to elucidate its biological relation to cystine.[13] Somewhat similar is the synthesis of *meso*-ωω′-bimethionine (I), an impurity normally present in methionine prepared by the Barger–Weichselbaum method. (I) is apparently the material formerly termed ψ-methionine, though the *dl*-compound is probably also present in this by-product. The structure of (I) was confirmed by conversion

[5] N. D. Cheronis *et al.*, *J. Org. Chem.*, 1941, **6**, 349, 467; A. F. Chadwick and E. Pacsu, *J. Amer. Chem. Soc.*, 1941, **63**, 2427.

[6] C. S. Marvel, *Org. Syn.*, 1941, **21**, 74, 60. *iso*Leucine is said to be biologically detectable in synthetic leucine; D. M. Hegsted and E. D. Wardwell, *J. Biol. Chem.*, 1944, **153**, 167.

[7] C. S. Marvel, *Org. Syn.*, 1940, **20**, 106.

[8] *Idem*, *ibid.*, 1941, **21**, 99.

[9] H. E. Carter and H. D. West, *ibid.*, 1940, **20**, 81.

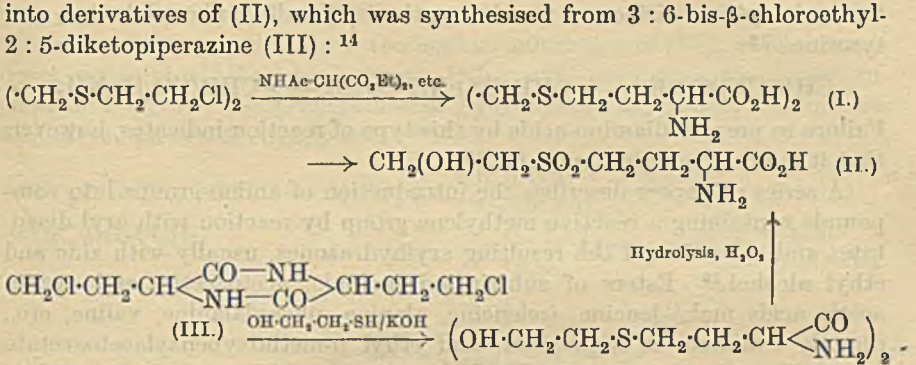
[10] R. E. Steiger, *ibid.*, 1942, **22**, 23.

[11] *Idem*, *ibid.*, p. 13.

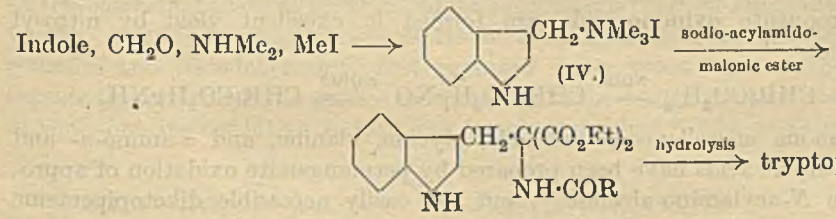
[12] *Idem*, *ibid.*, 1944, **24**, 9.

[13] G. W. Kilmer and V. du Vigneaud, *J. Biol. Chem.*, 1944, **154**, 247.

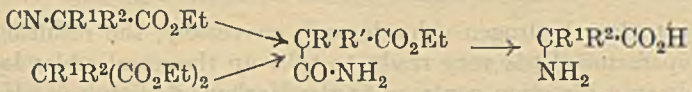
into derivatives of (II), which was synthesised from 3 : 6-bis-β-chloroethyl-2 : 5-diketopiperazine (III) : [14]



An attractive modification of the malonic ester method, which is, however, probably of only limited application, provides what seems to be the best of the available syntheses of tryptophan. Quaternary salts containing a group of the benzyl type are known to behave as *C*-alkylating agents. Gramine methiodide (IV) is of this kind and as gramine is formed " under physiological conditions," tryptophan is easily accessible in a yield of 45% based on the consumption of indole; [15] this type of reaction can also be carried out with malonic or benzamidomalonic ester : [16]



The degradation of ester-hydrazides or ester-amides of substituted malonic acids by the Curtius, Hofmann or equivalent reaction has long been a potentially useful route to amino-acids or their near derivatives. Hitherto the preparation of the malonic derivatives has been difficult, but several syntheses of this type have been accomplished from malonic and cyano-acetic ester : [17]



$R_1 = Et, R_2 = Et$; $R_1 = Et, R_2 = Pr$; $R_1, R_2 =$ *iso*butyl; $R_1, R_2 =$ *iso*amyl.

The inverse series by which the ester group of an alkyl cyanoacetate is degraded to NH_2 by the Curtius method whilst the CN ultimately provides

[14] H. R. Snyder, E. E. Howe, G. W. Cannon, and M. A. Nyman, *J. Amer. Chem. Soc.*, 1943, **65**, 2211.

[15] H. R. Snyder and C. W. Smith, *ibid.*, 1944, **66**, 350.

[16] N. F. Albertson, S. Archer, and C. M. Suter, *ibid.*, p. 500.

[17] Y. T. Huang *et al.*, *J. Chinese Chem. Soc.*, 1941, **8**, 201; Lianghi *et al.*, *ibid.*, 1942, **9**, 1, 14, 31.

the carboxyl is satisfactory for the synthesis of valine, phenylalanine, and tyrosine : [18]

$$CHR(CN)\cdot CO_2Et \longrightarrow CHR(CN)\cdot NH\cdot CO_2Et \longrightarrow CHR(CO_2H)\cdot NH_2$$

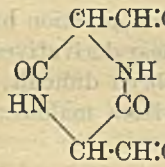
Failure to prepare diamino-acids by this type of reaction indicates, however, that it is not generally applicable.[18]

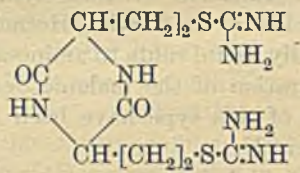
A series of papers describes the introduction of amino-groups into compounds containing a reactive methylene group by reaction with aryl diazotates and reduction of the resulting arylhydrazones, usually with zinc and ethyl alcohol.[19] Esters of substituted malonic, acetoacetic, and cyanoacetic acids make leucine, *iso*leucine, alanine, phenylalanine, valine, etc., directly available in high yield, and ethyl *p*-methoxybenzylacetoacetate eventually affords tyrosine. Similarly, ethyl *cyclo*hexanone-2-carboxylate ultimately affords α-aminopimelic acid. This route is similar to that *via* nitrosoacetoacetic esters where the intermediate α-oximino-esters may be reduced catalytically to α-amino-esters; alanine, α-aminobutyric acid, norvaline, norleucine, *iso*leucine, phenylalanine, tyrosine, aspartic and glutamic acids are among those recently prepared in this manner.[20] In this type of reaction alkylmalonic acids are similarly reactive, though probably less convenient than the esters; it is, however, of practical interest that, whereas alkyl nitrites are sometimes troublesome for this purpose, the requisite oximino-acids are formed in excellent yield by nitrosyl chloride : [21]

$$CHR(CO_2H)_2 \xrightarrow{NOCl} CHR(CO_2H)\cdot NO \xrightarrow{Sn/HCl} CHR(CO_2H)\cdot NH_2$$

Among miscellaneous methods, glycine, alanine, and α-amino-*n*- and -*iso*-butyric acids have been prepared by permanganate oxidation of appropriate *N*-acylamino-alcohols,[22] and the easily accessible diketopiperazine (V) is a possible source of several amino-acids. (III) exhibits an unexpected

(V.)





(VI.)

tendency to lose hydrogen chloride and, conversely, the resulting diketodivinylpiperazine (V) is very ready to take up the usual addenda, though invariably in a direction contrary to the Markownikoff rule.[23] Hydrolysis of the methylthiol and hydrogen sulphide addition products, for example,

[18] P. E. Gagnon, R. Gaudry, and F. E. King, *J.*, 1944, 13.

[19] V. V. Feofilaktov *et al.*, *Compt. rend. Acad. Sci. U.R.S.S.*, 1939, **24**, 755, 759; *J. Gen. Chem. Russia*, 1940, **10**, 247, 258, 260; 1943, **13**, 358, 363, 457.

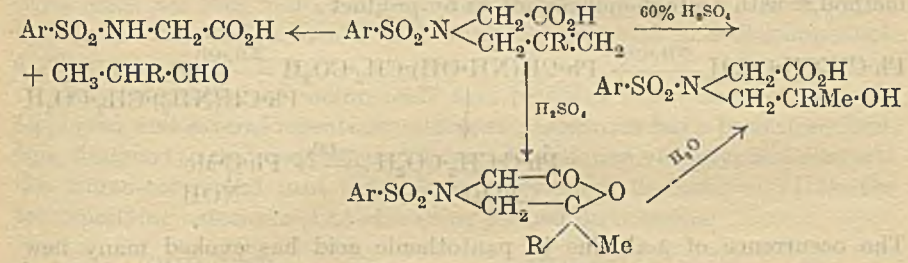
[20] K. E. Hamlin and W. H. Hartung, *J. Biol. Chem.*, 1942, **145**, 349.

[21] A. S. Onishchenko, *J. Gen. Chem. Russia*, 1941, **11**, 197.

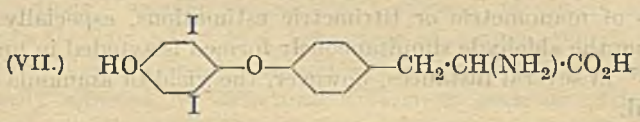
[22] J. H. Billman and E. E. Parker, *J. Amer. Chem. Soc.*, 1943, **65**, 761, 2455; 1944, **66**, 538.

[23] H. R. Snyder *et al.*, *ibid.*, 1944, **66**, 511, 1000, 1002.

gave methionine and homocysteine respectively and noteworthy also is the synthesis of the second acid *via* the bisthiuronium salt of (VI), which breaks down on mild alkaline hydrolysis. Other syntheses of some degree of generality include the preparation of hydroxyalkylamino-acid derivatives from arysulphonylalkenylglycines : [24]



The reaction is sometimes attended by complications such as fission to aldehydes, which are, however, susceptible to electronic interpretation. In this connection *N*-substituted alanines (like alanine itself) are said to be formed from acetaldehyde cyanohydrin and primary or *sec.*-amines (or ammonia),[25] and a very similar facile hydrolysis of a nitrile group makes sarcosine easily available.[26] Several specific preparations are also noted.[27] Of the individual acids, thyroxine and its biosynthesis [28] are of much interest to the organic chemist in view of its *in vitro* formation from iodinated proteins and di-iodotyrosine, first reported some years ago. Although repeatedly confirmed, the precise mechanism of this change remains obscure except that its oxidative nature has been established.[29] In addition to



3 : 5-di-iodo-*dl*-thyronine itself, the 3′ : 5′- and later the 2′ : 6′-isomeride (VII) have been made,[30] the latter by the device of converting NH_2 into N_2BF_4, which was in turn replaced by warming with acetic acid. The projected isomeride of thyroxine was, however, not obtained. In this connection 3-iodotyrosine has been prepared from 3-aminotyrosine by the diazonium salt method.[30b]

[24] W. Cocker, *J.*, 1943, 373. [25] U.S.P. 2,328,940.

[26] E. Schütte, *Z. physiol. Chem.*, 1943, **279**, 61.

[27] Glycine *via* its nitrile from aminomethanesulphonic acid; U.S.P. 2,346,547. β-Phenylalanine from benzenediazonium chloride and acrylonitrile; R. Gaudry, *Laval Med.*, 1944, **9**, 412. *dl*-1-Methylhistidine; W. Sakami and D. W. Wilson, *J. Biol. Chem.*, 1944, **154**, 215.

[28] C. R. Harington, *J.*, 1944, 193; *Proc. Roy. Soc.*, 1944, *B*, **132**, 223.

[29] A. E. Barkdoll and W. F. Ross, *J. Amer. Chem. Soc.*, 1944, **66**, 898.

[30] (*a*) P. Block and G. Powell, *ibid.*, 1942, **64**, 1070; C. Niemann and G. E. McCasland, *ibid.*, 1944, **66**, 1870; Synthesis of 3 : 5-di-iodo- and 3 : 5 : 3′ : 5′-tetraiodo-4-(4′-hydroxyphenoxy)hippuric acid, *ibid.*, p. 1984. (*b*) C. R. Harington and R. V. Pitt Rivers, *Biochem. J.*, 1944, **38**, 320.

Except β-alanine, β-amino-acids are of relatively little interest, though they can be made conveniently by adding ammonia to αβ-unsaturated acids at 125—135°[31] or by the interaction of aldehyde ammonias with malonic acid, some undergoing facile pyrimidine and glyoxalidone syntheses.[32] β-Amino-β-phenylpropionic acid is readily prepared by modifying an earlier method,[33] with acetophenoneoxime as by-product:

$$\mathrm{Ph{\cdot}CH{:}CH{\cdot}CO_2H} \xrightarrow{\mathrm{NH_2{\cdot}OH}} \mathrm{Ph{\cdot}CH(NH{\cdot}OH){\cdot}CH_2{\cdot}CO_2H} \xrightarrow{\mathrm{NH_2{\cdot}OH}} \mathrm{Ph{\cdot}CH(NH_2){\cdot}CH_2{\cdot}CO_2H}$$

$$\mathrm{Ph{\cdot}C(NOH){\cdot}CH_2{\cdot}CO_2H} \xrightarrow{-\mathrm{CO_2}} \mathrm{Ph{\cdot}C(NOH){\cdot}Me}$$

The occurrence of β-alanine in pantothenic acid has evoked many new preparations, mostly described in patents;[34] one of the most convenient is the smooth hydrogenation of ethyl cyanoacetate in acetic–sulphuric acid, a noteworthy reaction in view of the usual difficulty of reducing aliphatic cyano-groups.[35]

Analytical Reactions of Amino-acids.

No attempt is made here to review purely formal quantitative methods, but it is perhaps permissible to mention quantitative applications of the ninhydrin reaction. Carbon dioxide, which is liberated within a few minutes, may be used on a micro- or macro-scale in estimating free α-amino-acids, titrimetrically or volumetrically.[36] Ammonia liberated can also form the basis of manometric or titrimetric estimations, especially when interference from the aldehyde simultaneously formed is avoided in presence of hydrazine;[37] in several instances, however, the yield of ammonia is less than theoretical.

Several qualitative reactions which have some degree of analytical application have come to light. For instance, certain amino-acids give fairly characteristic chromogens on heating,[38] and diamino-acids and a few

[31] K. Lang and F. Adickes, *Z. physiol. Chem.*, 1941, **269**, 236.

[32] V. M. Rodionov and V. A. Zvorykina, *Bull. Acad. Sci. U.R.S.S.*, 1943, 216.

[33] R. E. Steiger, *Org. Syn.*, 1942, **22**, 26.

[34] From acrylonitrile–ammonia; U.S.P. 2,335,997. From bis-β-cyanoethylamine–ammonia; U.S.P. 2,334,163. From thiodihydroacrylonitrile–ammonia; U.S.P. 2,335,653. From acrylic esters–ammonia; Canadian P. 417,270. From ethylenecyanohydrin–ammonia; Canadian P. 417,165. From β-alkoxypropionitriles–ammonia; U.S.P. 2,335,605; cf also 2,336,067. Reduction of nitropropionic esters; B.P. 551,990.

[35] F. Weygand, *Ber.*, 1941, **74**, 256.

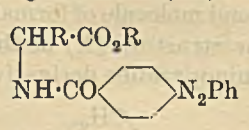
[36] D. D. Van Slyke *et al.*, *J. Biol. Chem.*, 1941, **141**, 627, 671; 1943, **150**, 231, 251; D. A. MacFadyen, *ibid.*, 1942, **145**, 387; M. S. Dunn *et al.*, *ibid.*, 1943, **151**, 241.

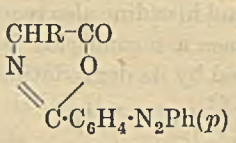
[37] D. A. MacFadyen, *ibid.*, 1944, **153**, 507; H. F. Schott, L. B. Rockland, and M. S. Dunn, *ibid.*, 1944, **154**, 397.

[38] H. Tauber, *J. Amer. Chem. Soc.*, 1944, **66**, 310.

others give phosphotungstates though of irregular composition.[39] Certain arylsulphonates are appearing to be of value in this connection; *e.g.*, histidine is separated from protein hydrolysates as its salt with 2 mols. of 3 : 4-dichlorobenzenesulphonic acid[40] and serine, arginine, lysine, and histidine can be individually precipitated by selected sulphonic acids.[41] Such acids are still more useful in isolating dipeptides, *e.g.*, from partly hydrolysed silk fibroin with 2 : 5-dibromobenzene- and 2 : 6-di-iodophenol-4-sulphonic acid.[42]

Chromatography of amino-acids and peptides[43] has been extensively employed and several essentially different procedures have been described. One, designed so that the preparation may be followed visually, first converts the amino-compound into the coloured acylamido-derivative (VIII), the corresponding azlactone (IX) also being formed on occasion.

(VIII.) 

 (IX.)

As formation of the latter entails racemisation of an optically active acid, the acylation is preferably done in pyridine.[44] Partition chromatography of acylamido-acids finds a typical use in the examination of hydrolysates of tyrocidin and gramicidin, an important result being the recognition of amino-acids of "unnatural" (*d*-) configuration.[45] Many applications of ordinary chromatography are purely empirical, though noteworthy for their use of unusual adsorbents[46] and for the characterisation of amino-acids by their "specific retardation volumes" of eluent under specified conditions.[47] The adsorption behaviour of amino-acids is markedly dependent on their betaine nature and on other polar groups present, and only secondarily affected by structural differences such as variation in chain configuration. So when the α-amino-group is masked by reaction with

[39] D. D. Van Slyke, A. Hiller, and R. T. Dillon, *J. Biol. Chem.*, 1942, **146**, 137. Other quantitative reactions : Glutamic acid converted quantitatively into pyrrolidonecarboxylic acid, H. S. Olcott, *ibid.*, 1944, **153**, 71; preparation and separation of *NN'*-dibenzoylcystine or its insoluble sodium salt from cysteine–lanthionine mixtures, A. Schöberl, *Ber.*, 1943, **76**, 964; oxidation of methionine, etc., to its periodide, T. F. Lavine, *J. Biol. Chem.*, 1943, **151**, 281; preliminary precipitation of histidine as its salt with nitroanilic acid, F. Vilallonga, *Rev. Soc. argent. Biol.*, 1943, **19**, 43.

[40] H. B. Vickery and J. K. Winternitz, *J. Biol. Chem.*, 1944, **156**, 211.

[41] W. H. Stein, S. Moore, G. Stamm, C. Y. Chou, and M. Bergmann, *ibid.*, 1941, **143**, 121.

[42] W. H. Stein, S. Moore, and M. Bergmann, *ibid.*, 1944, **154**, 191.

[43] See *Ann. Reports*, 1942, 237.

[44] P. Karrer, R. Keller, and G. Szönyi, *Helv. Chim. Acta*, 1943, **26**, 38.

[45] A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, 1943, **37**, 79, 86, 313.

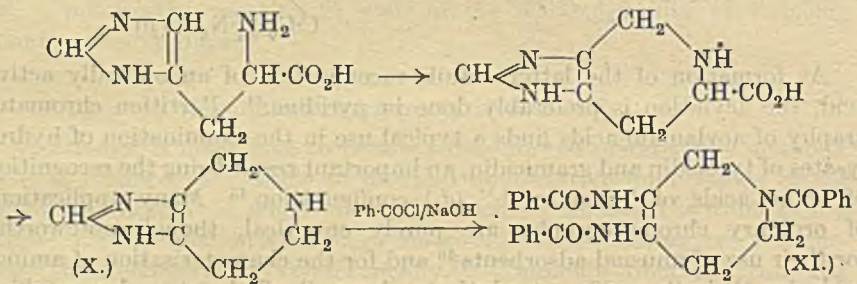
[46] Potato starch, R. L. M. Synge, *ibid.*, 1944, **38**, 285; charcoal-cellulose, J. L. Wachtel and H. G. Cassidy, *J. Amer. Chem. Soc.*, 1943, **65**, 665.

[47] A. Tiselius, *Arkiv Kemi, Min., Geol.*, 1941, *B*, **15**, No. 6

formaldehyde, adsorption becomes apolar and finer separation is effected.[48] Again, " basic " amino-acids in neutral solution are readily taken up by alumina, whereas " neutral " amino-acids remain unadsorbed; on acid-washed alumina, adsorption is " anionotropic " and " acid " amino-acids are retained, so this behaviour is of preparative value.[49]

Further Reactions of Amino-acids.

In spite of intensive study by polarimetric, potentiometric, and other means, the reaction between amino-acids and formaldehyde continues to give discordant results. Most workers agree that the prime reaction is centred round the α-amino-group and leads reversibly to an aldimine or methylol. With proteins and peptides further reaction is believed to be due to formation of methylene bridges,[50] but amino-acids such as glutamic acid, lysine, and histidine also react with a second molecule of formaldehyde.[51] In the last case a second ring is formed, the structure (X) of the product being indicated by its degradation to the diaminopyridine derivative (XI) :[52]



When α-amino-esters are treated with carbon dioxide in cold ether, they are converted into amino-ester salts of *N*-carboxyamino-esters.[53] The reaction is similar to that between carbon disulphide and amines and is a modification of the Siegfried reaction between amino-acids in the anionic form, $NH_3^+ \cdot CHR \cdot CO_2^-$, and carbon dioxide, the products being in some cases usefully isolated as their salts, $\begin{matrix} CHR \cdot CO_2 \\ NH-CO_2 \end{matrix} > Ba$. A similar reactivity of the anion is used to prepared guanidino-acids in excellent yield by reaction in ammoniacal solution with *S*-methyl*iso*thiourea, and the same method can be

[48] V. H. Cheldelin and R. J. Williams, *J. Amer. Chem. Soc.*, 1942, **64**, 1513; G. Schramm and J. Primosigh, *Ber.*, 1943, **76**, 373.

[49] T. Wieland, *Z. physiol. Chem.*, 1942, **273**, 24; *Naturwiss.*, 1942, **30**, 374; *Die Chemie*, 1943, **56**, 213; F. Turba and M. Richter, *Ber.*, 1942, **75**, 340; F. Turba, M. Richter, and F. Kuchar, *Naturwiss.*, 1943, **31**, 508; T. Wieland and L. Wirth, *Ber.*, 1943, **76**, 823; R. K. Cannan, *J. Biol. Chem.*, 1944, **152**, 401; A. C. Kibrick, *ibid.*, p. 411.

[50] S. Fiala, *Naturwiss.*, 1943, **31**, 370; H. Nitschmann and H. Hadorn, *Helv. Chim. Acta*, 1944, **27**, 299; R. L. Wormall and M. A. G. Kaye, *Nature*, 1944, **153**, 525.

[51] E. H. Frieden, M. S. Dunn, and C. D. Coryell, *J. Physical Chem.*, 1943, **47**, 10, 85, 118.

[52] A. Neuberger, *Biochem. J.*, 1944, **38**, 309.

[53] M. Frankel and E. Katchalski, *J. Amer. Chem. Soc.*, 1943, **65**, 1670.

used to "guanylate" free amino-groups in peptides and proteins.[54] In certain instances (*e.g.*, sarcosine ⟶ creatine) *O*-methyl*iso*urea behaves as a guanylating agent and the guanidino-group can also be introduced indirectly, *e.g.* :

$$NH(CH_2{\cdot}CO_2Me)_2 \xrightarrow{NH_2{\cdot}CN} \begin{matrix} NH{\cdot}C(:NH){\cdot}N{\cdot}CH_2{\cdot}CO_2Me \\ CO\text{———}CH_2 \end{matrix}$$

$$\downarrow BrCN$$

$$CN{\cdot}N(CH_2{\cdot}CO_2Me)_2 \xrightarrow{NH_3} \begin{matrix} NH{\cdot}C(:NH){\cdot}N{\cdot}CH_2{\cdot}CO{\cdot}NH_2 \\ CO\text{———}CH_2 \end{matrix}$$

Among other reactions of the α-amino-group, the transamination reaction is of special interest to the biochemist and organic chemist : [55]

$$\begin{matrix} CHR{\cdot}CO_2H \\ NH_2 \\ COR'{\cdot}CO_2H \end{matrix} \longrightarrow \begin{matrix} CHR{\cdot}CO_2H \\ N \\ \| \\ CR'{\cdot}CO_2H \\ \text{(XII.)} \end{matrix} \longrightarrow \begin{matrix} CR{\cdot}CO_2H \\ \| \\ N \\ CHR'{\cdot}CO_2H \\ \text{(XIII.)} \end{matrix} \longrightarrow$$

$$\begin{matrix} COR{\cdot}CO_2H \\ NH_2 \\ CHR'{\cdot}CO_2H \end{matrix} \longrightarrow \begin{matrix} CO_2 + R{\cdot}CHO \\ NH_2 \\ CHR'{\cdot}CO_2H \end{matrix}$$

The natural reaction is enzymatic, and the *in vitro* one is conveniently carried out in aqueous solution at 100°. The latter is apparently not limited to keto-acids, for alanylalanine has been so prepared,[56] and a

$$Me{\cdot}CO{\cdot}CO{\cdot}NH{\cdot}CHMe{\cdot}CO_2H \xrightarrow{Ph{\cdot}CH(NH_2){\cdot}CO_2H} NH_2{\cdot}CHMe{\cdot}CO{\cdot}NH{\cdot}CHMe{\cdot}CO_2H$$

synthesis of peptides from α-keto-acids drawing upon ammonia or other amino-acids for a source of nitrogen may be envisaged :

$$R{\cdot}CO{\cdot}CO_2H \longrightarrow R{\cdot}CH(NH_2){\cdot}CO_2H \xrightarrow{R'{\cdot}CO{\cdot}CO_2H} R'{\cdot}CO{\cdot}CO{\cdot}NH{\cdot}CO{\cdot}CHR{\cdot}CO_2H, \text{ etc.}$$

Such a hypothesis has the advantage over the synthesis from amino-acids which is usually assumed that it explains how isotopic nitrogen in a dietary amino-acid may appear combined in a different amino-acid in the body proteins. The above formulation of the transamination reaction explains, if it be assumed that the intermediate Schiff bases (XII), (XIII) can undergo decarboxylation, the occasional formation of two aldehydes R·CHO and R'·CHO. There is, however, no direct evidence for such interaction of α-amino-acids and α-keto-acids, although the synthesis of octopin (XIV) provides indirect evidence :

$$\begin{matrix} \text{Arginine} \\ \text{+ pyruvic acid} \end{matrix} \longrightarrow \begin{matrix} CH_2{\cdot}NH{\cdot}C(NH){\cdot}NH_2 \\ CH_2 \\ CH{\cdot}N{:}CMe{\cdot}CO_2H \\ CO_2H \end{matrix} \xrightarrow{H_2} \begin{matrix} CH_2{\cdot}NH{\cdot}C(NH){\cdot}NH_2 \\ CH_2 \\ CH{\cdot}NH{\cdot}CHMe{\cdot}CO_2H \\ CO_2H \\ \text{(XIV.)} \end{matrix}$$

[54] E. Schütte, *Z. physiol. Chem.*, 1943, **279**, 52, 59.

[55] Reviews : T. Wieland, *Die Chemie*, 1942, **55**, 147; R. M. Herbst, *Advances in Enzymology*, 1944, **4**, 75.

[56] R. M. Herbst and D. Shemin, *J. Biol. Chem.*, 1943, **147**, 541.

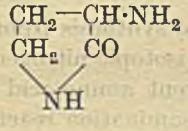
The postulated Schiff base system is not completely comparable with simple azomethine systems studied from an electronic standpoint some years ago, since in transamination, reaction is unidirectional and accompanied by elimination of carbon dioxide. Nevertheless variation of rate with substitution in the phenyl group R follows that anticipated from their known electronic behaviour, although the superiority of *o*- over *p*-substitution is curious.[57]

$$o\text{-Cl} > o\text{-OMe} > o\text{-OH} > p\text{-Cl} > p\text{-OMe} > p\text{-OH}$$

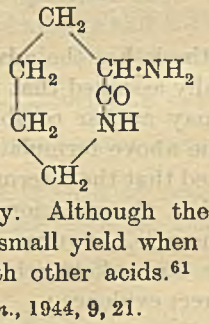
More precise evaluation of the hypothesis is complicated by the fact that changing substitution influences the course, *i.e.*, the relative composition of the mixture of aldehydes produced, as well as the rate of reaction. Space permits only reference[58] to several other reactions of interest and the remainder of this Report is devoted to a consideration of dehydration products of amino-acids and peptides.

It has long been known that glycine ethyl ester undergoes self-condensation to glycine anhydride and, in ether, for example, to " biuret base," $NH_2{\cdot}CH_2{\cdot}CO{\cdot}[NH{\cdot}CH_2{\cdot}CO]_2{\cdot}NH{\cdot}CH_2{\cdot}CO_2Et$. The tetrapeptide does not represent the limit of condensation, for keeping glycine esters in various organic solvents at temperatures up to the b. p. gives polycondensation products containing 12—30 glycine residues. On heating to 130° several undergo further condensation to give chains of up to 110 glycine units. Alanine ester behaves similarly, though the tendency to condensation is less.[59] " Anhydrides " of basic amino-acids prepared from esters have been assumed to be diketopiperazines, but are now known to consist in part of unimolecular heterocyclic amines. Methyl $\alpha\gamma$-diamino-*n*-butyrate and lysine ester thus give 3-amino-pyrrolidone (XV) and -homopiperidone (XVI) respectively,[60] and the similar formation of 3-aminopiperidone from

(XV.)



(XVI.)



ornithine derivatives was known previously. Although the amino-acids are more stable, nicotinamide is formed in small yield when asparagine is heated with glutamic acid and perhaps with other acids.[61] Diketopiper-

[57] E. K. Harvill and R. M. Herbst, *J. Org. Chem.*, 1944, **9**, 21.

[58] " Reverse aldol reaction " of certain hydroxybetaines, *e.g.*,

$$CHMe(OH){\cdot}CH(\overset{+}{N}Me_3){\cdot}\overset{-}{C}O_2 \longrightarrow Me{\cdot}CHO + CH_2(\overset{+}{N}Me_3){\cdot}\overset{-}{C}O_2:$$

H. D. Dakin, *J. Biol. Chem.*, 1941, **140**, 847. Formation and reactions of *N*-hydroxy-amino-acids : R. E. Steiger, *J. Biol. Chem.*, 1944, **153**, 691. Preparation of diazoacetic ester : E. B. Womack and A. B. Nelson, *Org. Syn.*, 1944, **24**, 56. Cystine and cysteine hydantoin : J. V. Karabinos and J. L. Szabo, *J. Amer. Chem. Soc.*, 1944, **66**, 649.

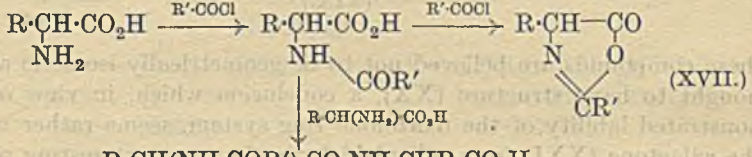
[59] M. Frankel and E. Katchalski, *J. Amer. Chem. Soc.*, 1942, **64**, 2264, 2268.

[60] D. W. Adamson, *J.*, 1943, 39.

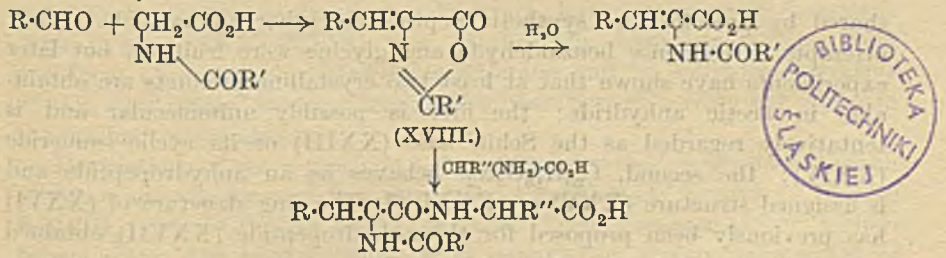
[61] J. R. Bovarnick, *J. Biol. Chem.*, 1943, **149**, 301; **151**, 467; 1944, **153**, 1.

azines are, however, formed when dipeptides are dehydrated in fused α-naphthol.[62]

Unexpectedly facile anhydride formation is often encountered in the acylation of amino-acids. For instance, the normal Baum method of benzoylation with benzoyl chloride and aqueous sodium hydroxide was for a period supplanted by acylation in bicarbonate solution until it was shown that mixed anhydrides between benzoic and benzamido-acids tend to be formed;[63] if the original amino-acids are optically active, they become racemised during hydrolysis. In such instances benzoylation is cleanly effected by using controlled concentrations of caustic alkali.[64] Part of the difficulty is caused by the formation of azlactones (XVII) and though their stability under normal acylating conditions is surprising, there is little doubt that sodium salts of 2-*p*-nitrophenylazlactones (oxazolones) are responsible for the Waser test for α-amino-acids.[65] Certainly, when α-amino-acids in general are treated with acyl halides in pyridine, the ensuing action is more than a simple acylation, depends on both the amino-acid and the nature of the acyl group, and includes reactions of the following kind :

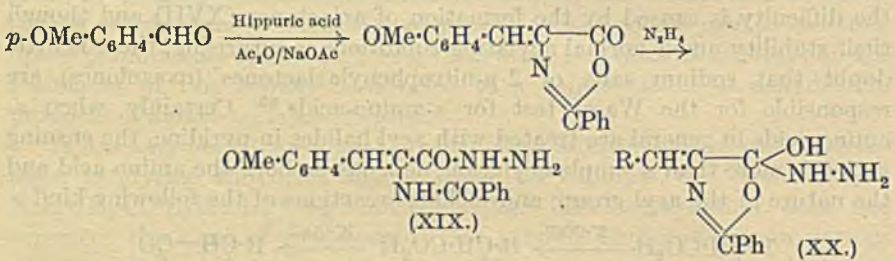


Generally aliphatic acid chlorides give better yields of simple acyl derivatives, and azlactones (XVII) and acyldipeptides arise from benzoyl chloride. It is pertinent to point out that acid chlorides of peptides may well be only hydrochlorides of weakly basic azlactones; as shown below, acylating reactions follow as well from azlactones as from orthodox acid chlorides. It is rarely, however, a recommended method for the preparation of acyldipeptides and indeed some amino-acids (serine, threonine, cystine, tyrosine) are unaffected by such treatment.[66] The simplest examples of azlactone–dipeptide formation occur as an extension of the Ploechl–Erlenmeyer azlactone synthesis of amino-acids :

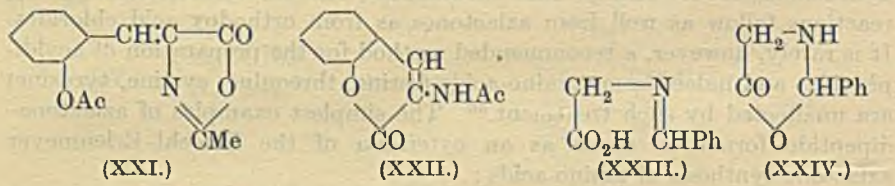


[62] N. Lichtenstein, *J. Amer. Chem. Soc.*, 1944, **66**, 1103.
[63] H. E. Carter and C. M. Stevens, *J. Biol. Chem.*, 1941, **138**, 628.
[64] R. E. Steiger, *J. Org. Chem.*, 1944, **9**, 396.
[65] P. Karrer and R. Keller, *Helv. Chim. Acta*, 1943, **26**, 50.
[66] H. E. Carter, P. Handler, and C. M. Stevens, *J. Biol. Chem.*, 1941, **138**, 619.

A derivative of glycine is condensed with, usually, an aromatic aldehyde. Ploechl failed to condense benzaldehyde with glycine itself and it was this fact which led to the use of hippuric acid and later of other derivatives such as hydantoin and glycine anhydride. The azlactone method has been used to prepare 4-arylidene azlactones (XVIII) and glycine derivatives in great variety, *e.g.*, from aceturic or hippuric acid and vanillin or its halogenated and other near derivatives.[67] They are hydrolysed normally to acylamidoferulic acids and thence to substituted pyruvic acids. The same method has been used to prepare *p*-methoxycinnamylideneglycine derivatives, *e.g.*, (XIX), some of which are obtainable in isomeric forms :



These compounds are believed not to be geometrically isomeric and one is thought to have structure (XX), a conclusion which, in view of the demonstrated lability of the oxazolone ring system, seems rather unlikely.[68] The azlactone (XXI) from salicylaldehyde shows an interesting reaction in that, on hydrolysis, the intermediate hydroxyacetamido-acid can have only a transient existence and passes into the acetamidocoumarin (XXII). Condensation with aromatic aldehydes is not limited to those glycine derivatives which ostensibly contain a reactive methylene group, but is



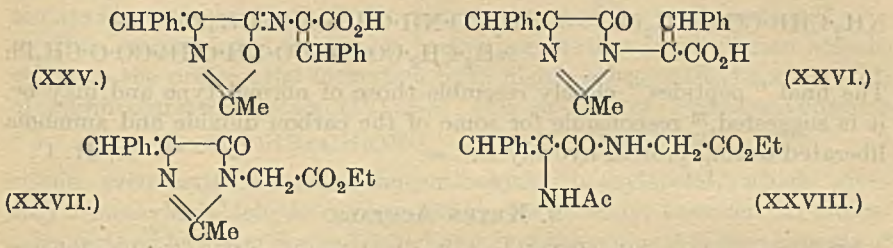
shared by proteins and synthetic peptides of other amino-acids. Early attempts to condense benzaldehyde and glycine were fruitless, but later experiments have shown that at least two crystalline products are obtainable in acetic anhydride; the first is possibly unimolecular and is tentatively regarded as the Schiff base (XXIII) or its cyclic isomeride (XXIV); the second, $C_{20}H_{16}O_3N_2$, behaves as an anhydropeptide and is assigned structure (XXV) or (XXVI).[69] The ring structure of (XXVI) has previously been proposed for the anhydropeptide (XXVII) obtained

[67] L. C. Raiford and C. H. Buurman, *J. Org. Chem.*, 1943, **8**, 466.

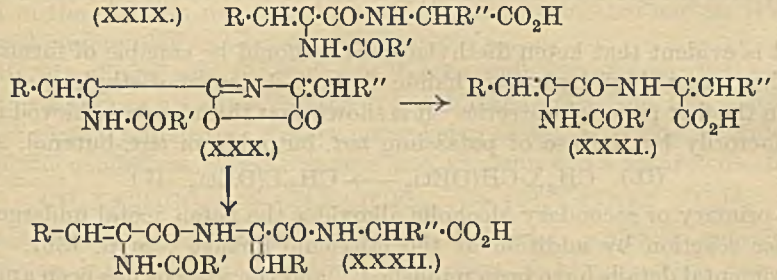
[68] M. Vanghelovici and I. Moise, *Soc. Chim. România Sect. Soc. romane Stiinte, Bul. Chim. purâ apl.*, 1941–2, (2), 3*A*, 85 (seen only in abstract).

[69] J. E. Tietzmann, D. G. Doherty, and M. Bergmann, *J. Biol. Chem.*, 1943, **151**, 387.

on heating acetyldehydrophenylalanylglycine ester (XXVIII) to 180° in a vacuum.[70]



Although, as was mentioned above, the formation of peptides from azlactones is variable, the formation of dehydropeptides (XXIX) comparable with (XXVIII) from 4-arylideneazlactones proceeds easily [71] and a large number have been so obtained. When R″ = H (XXIX), the acylpeptides yield doubly unsaturated azlactones (XXX), which are hydrolysable to diacylamino-compounds (XXXI), and the process may be repeated as in (XXXII)



to give tetra- and penta-peptides. Some of these dehydropeptides exist in stereoisomeric forms. When the azlactone of acetyldehydrophenylalanyldehydrophenylalanine (XXX; R = Ph, R′ = Me) is heated with aqueous pyridine, it undergoes a remarkable rearrangement to yield an acidic anhydropeptide, perhaps of structure (XXV) or (XXVI). This and similar products are moderately stable to mineral acid, unlike other anhydrides of acylated amino-acids (such as those from benzoylserine methyl ester and benzoylglycineamide, which, however, are perhaps oxazole derivatives). (XXV—XXVI) was catalytically hydrogenated to yield one form of acetylphenylalanylphenylalanine and an anhydro-derivative of this, also acidic, thus confirming the liberation of a carboxyl group in the original rearrangement.

Restrictions of space preclude a discussion of orthodox peptides, though attention may be directed to a review.[72] A number of " peptides " of type $NH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CO \cdot NH \cdot CHR \cdot CO_2H$ have been prepared from aminobenzyl esters (the benzyl group being removed at a convenient point of

[70] C. Graenacher and M. Mahler, *Helv. Chim. Acta*, 1927, **10**, 246.

[71] J. E. Tietzmann, D. G. Doherty, and M. Bergmann, *J. Biol. Chem.*, 1943, **147**, 617.

[72] M. Bergmann and J. S. Fruton, *Advances in Enzymology*, 1941, **1**, 63.

hydrogenation), by conversion into hydantoic acids with potassium cyanate, reaction with chloroacetyl chloride, and amination :

$$NH_2\cdot CHR\cdot CO\cdot O\cdot CH_2Ph \longrightarrow NH_2\cdot CO\cdot NH\cdot CHR\cdot CO\cdot O\cdot CH_2Ph \longrightarrow NH_2\cdot CH_2\cdot CO\cdot NH\cdot CO\cdot NH\cdot CHR\cdot CO\cdot O\cdot CH_2Ph$$

The final " peptides " closely resemble those of normal type and may be, it is suggested,[73] responsible for some of the carbon dioxide and ammonia liberated during protein hydrolysis. A. H. C.

3. Keten Acetals.

Some years ago, the Scheibler school claimed to have isolated keten acetals as intermediate products in the Claisen condensation of simple esters,[1] but other investigators [2] failed to substantiate those experiments. More recently, S. M. McElvain and his collaborators have evolved two general methods for the preparation of keten acetals, and have embarked upon a thorough investigation of their properties,[3–16] which are such as to leave no doubt that the products described by Scheibler were not compounds of this type.

It is evident that keten diethylacetal (I) should be capable of formation by the removal of hydrogen halide from a halogeno-diethylacetal (II), and in the first paper of the series [3] it is shown that this may be achieved most satisfactorily by the use of potassium *tert.*-butoxide in *tert.*-butanol, since

$$(II.)\quad CH_2X\cdot CH(OEt)_2 \longrightarrow CH_2{:}C(OEt)_2\quad (I.)$$

with primary or secondary alcoholic alkoxides the keten acetal undergoes a further reaction by addition at the ethylenic linkage (see p. 136). Full experimental details have been published,[17] and the reagent has been applied to the preparation of bromoketen diethylacetal,[4] chloro-, dichloro-, and dibromo-keten diethylacetals,[5] and to keten di-*n*-propyl-, di*iso*butyl-, and

[73] A. H. Corwin and C. I. Damerel, *J. Amer. Chem. Soc.*, 1943, **65**, 1974.

[1] H. Scheibler and H. Ziegner, *Ber.*, 1922, **55**, 789; H. Scheibler, E. Marhenkel, and R. Nikolić, *Annalen*, 1927, **458**, 21; H. Scheibler, *Ber.*, 1933, **66**, 428; *J. Amer. Chem. Soc.*, 1933, **55**, 425. Compare G. A. R. Kon, *Ann. Reports*, 1934, **31**, 200.

[2] J. M. Snell and S. M. McElvain, *J. Amer. Chem. Soc.*, 1933, **55**, 416, 429; F. Adickes and M. Meister, *Ber.*, 1935, **68**, 2191.

[3] F. Beyerstedt and S. M. McElvain, *J. Amer. Chem. Soc.*, 1936, **58**, 529.

[4] *Idem, ibid.*, 1937, **59**, 2266.

[5] A. Magnani and S. M. McElvain, *ibid.*, 1938, **60**, 2210.

[6] P. R. Johnson, H. M. Barnes, and S. M. McElvain, *ibid.*, 1940, **62**, 964.

[7] H. M. Barnes, D. Kundiger, and S. M. McElvain, *ibid.*, p. 1281.

[8] P. M. Walters and S. M. McElvain, *ibid.*, p. 1482.

[9] S. M. McElvain and D. Kundiger, *ibid.*, 1942, **64**, 254.

[10] S. M. McElvain and H. Cohen, *ibid.*, p. 260.

[11] S. M. McElvain and P. M. Walters, *ibid.*, p. 1059.

[12] S. M. McElvain, R. L. Clarke, and G. D. Jones, *ibid.*, p. 1966.

[13] S. M. McElvain, H. I. Anthes, and S. H. Shapiro, *ibid.*, p. 2525.

[14] S. M. McElvain and A. Jelinek, *ibid.*, 1943, **65**, 2236.

[15] S. M. McElvain and J. W. Langston, *ibid.*, p. 2239.

[16] S. M. McElvain and E. J. Engelhardt, *ibid.*, 1944, **66**, 1077.

[17] S. M. McElvain and D. Kundiger, *Org. Synth.*, 1943, **23**, 45.

di*iso*amyl-acetals.[11] Attempts to prepare keten diallyl- and dibenzyl-acetals resulted in the formation of allyl allylacetate and benzyl *o*-tolyl-acetate respectively, probably by rearrangement of the keten acetals.[13]

This method is not applicable to the preparation of alkylketen acetals, owing to the preferential formation from higher α-bromoalkyl acetals (III) of αβ-unsaturated acetals (IV);[8, 12] this αβ-loss of hydrogen bromide

$$\text{(III.)}\quad R{\cdot}CH_2{\cdot}CHBr{\cdot}CH(OEt)_2 \longrightarrow R{\cdot}CH{:}CH{\cdot}CH(OEt)_2 \quad \text{(IV.)}$$

occurs even with α-bromo*iso*butyraldehyde diethylacetal, which gives only α-methylacraldehyde diethylacetal. Fortunately, however, an alternative method has been devised, which involves the removal of the elements of alkyl hypobromite from an α-bromo-ortho-ester by treatment with sodium in boiling benzene :

$$R{\cdot}CHBr{\cdot}C(OEt)_3 \longrightarrow R{\cdot}CH{:}C(OEt)_2$$

Keten, methylketen,[8] *n*-propylketen, and *iso*propylketen diethylacetals,[12] and also keten dimethylacetal,[13] have been prepared by this route. The alkylketen acetals are stable to potassium *tert.*-butoxide,[8, 18] which shows that in the reaction mentioned above, the αβ-unsaturated acetals (IV) are formed preferentially and not by rearrangement.

H. Staudinger and G. Rathsam [19] prepared phenylketen diethylacetal by thermal decomposition of ethyl phenylorthoacetate, but this method is not of general application,[13] since many keten acetals are unstable under the pyrolytic conditions, and decompose into an olefin and a saturated ester :

$$CH_2X{\cdot}C(OEt)_3 \longrightarrow CHX{:}C(OEt)_2 \longrightarrow CH_2X{\cdot}CO_2Et + C_2H_4$$

A noteworthy exception is keten dimethylacetal, which is remarkably stable to heat. The intermediate formation of keten acetals has been demonstrated [13] by performing the pyrolysis of the ortho-ester in the presence of phenol, which reacts additively with the keten acetal to form unstable mixed ortho-esters, which in turn decompose into phenyl alkyl ethers. With a stable mixed ortho-ester (V) which may be conveniently prepared by the reaction of a keten acetal (VI) with an alcohol, the pyrolysis yields the original simple acetal (VI) together with the mixed acetal (VII). This is proved by incorporating phenol in the reaction mixture, whereupon the unstable mixed ortho-esters (VIII) and (IX) are formed, which decompose to give a mixture of esters and ethers.

$$\underset{\text{(VI.)}}{CH_2{:}C(OR)_2} \underset{+\,R'OH}{\overset{-\,R'OH}{\rightleftharpoons}} \underset{\text{(V.)}}{CH_3{\cdot}C(OR)_2{\cdot}OR'} \xrightarrow{-\,ROH} \underset{\text{(VII.)}}{CH_2{:}C(OR){\cdot}OR'}$$

$$\downarrow +\,PhOH \qquad\qquad \downarrow +\,PhOH$$

$$\underset{\text{(VIII.)}}{CH_3{\cdot}C(OR)_2{\cdot}OPh} \qquad \underset{\text{(IX.)}}{CH_3{\cdot}C(OR)(OR'){\cdot}OPh}$$

$$\downarrow \qquad\qquad \downarrow$$

$$CH_3{\cdot}CO_2R + Ph{\cdot}OR \qquad CH_3{\cdot}CO_2R + Ph{\cdot}OR' + CH_3{\cdot}CO_2R' + Ph{\cdot}OR$$

[18] E. Rothstein, *J.*, 1940, 1558.

[19] *Helv. Chim. Acta*, 1922, **5**, 645.

Keten acetals show wide differences in reactivity, according to the nature of the substituent groups. The halogenoketen acetals, for example, are relatively stable, whereas keten diethylacetal is exceedingly sensitive to traces of acid, and polymerises if kept in acid-washed glassware; this polymerisation is also accelerated by heat and by inorganic halides.[6] The addition of a little cadmium chloride gives a mixed product, which contains the dimer (X) and a white solid polymer (XI; n, *ca.* 21). The latter probably

$CH_3{\cdot}C(OEt)_2{\cdot}CH{:}C(OEt)_2$ (X.) $\qquad$ $CH_3{\cdot}C(OEt)_2{\cdot}[CH_2{\cdot}C(OEt)_2]_n{\cdot}CH_2{\cdot}C(OEt)_3$ (XI.)

contains a number of cross linkages, formed by intermolecular loss of ethanol, but the high ethoxyl content indicates that this cannot be extensive. On acid hydrolysis it gives carbon dioxide and a dark red glass, probably (XII; n, *ca.* 21), which is soluble without decomposition in alkali, and oxidised by

(XII.) $CH_3{\cdot}CO{\cdot}[CH{:}C(OH)]_n{\cdot}CH_3$ $\qquad$ $CH_2{=}C(OEt)(OEt)$ (XIII.)

permanganate to carbon dioxide and acetic acid. The polymerisation appears to be entirely of the head-to-tail type, and this is probably due to the strength of the anionoid centre in its hetero-enoid structure (XIII).

In the presence of hydrogen fluoride, keten diethylacetal undergoes a cyclic trimerisation to yield 1 : 1 : 3 : 3 : 5 : 5-hexaethoxy*cyclo*hexane, together with the dimer (X). The trimer, which may be looked upon as the tri-acetal of *cyclo*hexane-1 : 3 : 5-trione, is stable to alkali but very sensitive to acids, and may be hydrolysed *via* phloroglucinol triethyl ether to phloroglucinol. Methylketen diethylacetal does not undergo this polymerisation, but reacts with hydrogen fluoride to give ethyl propionate and ethyl fluoride, probably *via* the addition product (XIV).

$$CH_3{\cdot}CH{:}C(OEt)_2 \longrightarrow \underset{\text{(XIV.)}}{CH_3{\cdot}CH_2{\cdot}CF(OEt)_2} \longrightarrow CH_3{\cdot}CH_2{\cdot}CO_2Et + EtF$$

The formation of addition products similar to (XIV) is very common in the chemistry of the keten acetals, and another example, involving the addition of phenol, has already been mentioned (p. 135). Considering this addition in a more general fashion, in the reaction of one mole of AB with one mole of keten diethylacetal, the primary product (XV) is often unstable and may decompose by loss of HB or EtB to give (XVI) or (XVII). The acetal (XVI) may then react with a further mole of AB to give (XVIII),

$$\underset{\text{(XVI.)}}{A{\cdot}CH{:}C(OEt)_2} \xleftarrow{-HB} \underset{\text{(XV.)}}{A{\cdot}CH_2{\cdot}CB(OEt)_2} \xrightarrow{-EtB} \underset{\text{(XVII.)}}{A{\cdot}CH_2{\cdot}CO_2Et}$$

$$\downarrow +AB$$

$$\text{(XVIII.)}\ A_2CH{\cdot}CB(OEt)_2 \xrightarrow{-EtB} \underset{\text{(XIX.)}}{A_2CH{\cdot}CO_2Et}$$

which by loss of EtB yields (XIX). A loss of HB from (XVIII), which would lead to further complications, has not so far been observed, but the

fragments HB and EtB are frequently acidic or alcoholic in nature, and will therefore react with further quantities of the keten acetal. In this way it is possible to explain the presence of many by-products.

TABLE I.

1 : 2-*Addition to Keten Diethylacetal.*

Reagent.	Primary adduct (frequently hypothetical).	Main secondary products.	References.
(*a*) H_2	$CH_3{\cdot}CH(OEt)_2$	—	3
(*b*) EtOH	$CH_3{\cdot}C(OEt)_3$	—	3
(*c*) HX (X = Cl; Br; $R{\cdot}CO_2$; PhO)	$CH_3{\cdot}CX(OEt)_2$	$CH_3{\cdot}CO_2Et$; EtX	3, 7
(*d*) NH_3	$CH_3{\cdot}C(NH_2)(OEt)_2$	$CH_3{\cdot}C(OEt){:}NH$; $CH_3{\cdot}CN$; $CH_3{\cdot}C(NH_2){:}NH$	7
(*e*) NH_2Ph	$CH_3{\cdot}C(NHPh)(OEt)_2$	$CH_3{\cdot}C(OEt){:}NPh$	7
(*f*) NHEtPh	$CH_3{\cdot}C(NEtPh)(OEt)_2$	$CH_3{\cdot}C(OEt){\cdot}NEtPh$	7
(*g*) Piperidine	$CH_3{\cdot}C(NC_5H_{10})(OEt)_2$	$CH_3{\cdot}C(NC_5H_{10})_2$	7
(*h*) *n*-C_4H_9Br	*n*-$C_4H_9{\cdot}CH_2{\cdot}CBr(OEt)_2$	*n*-$C_4H_9{\cdot}CH_2{\cdot}CO_2Et$	9
(*i*) $CH_3{\cdot}COCl$	$CH_3{\cdot}CO{\cdot}CH_2{\cdot}CCl(OEt)_2$	$CH_3{\cdot}C(O{\cdot}CO{\cdot}CH_3){:}CH{\cdot}CO_2Et$	9
(*j*) $CH_2(COPh)_2$	$CH_3{\cdot}C(O{\cdot}CPh{:}CH{\cdot}COPh)(OEt)_2$	—	7
(*k*) $CH_3{\cdot}CO{\cdot}CH_2{\cdot}CO_2Et$	$CH_3{\cdot}C(OEt)_2{\cdot}CH(CO{\cdot}CH_3){\cdot}CO_2Et$	$CH_3{\cdot}C(OEt){:}C(CO{\cdot}CH_3){\cdot}CO_2Et$	7
(*l*) $CH_2(CO_2Et)_2$	$CH_3{\cdot}C(OEt)_2{\cdot}CH(CO_2Et)_2$	$CH_3{\cdot}C(OEt){:}C(CO_2Et)_2$	7

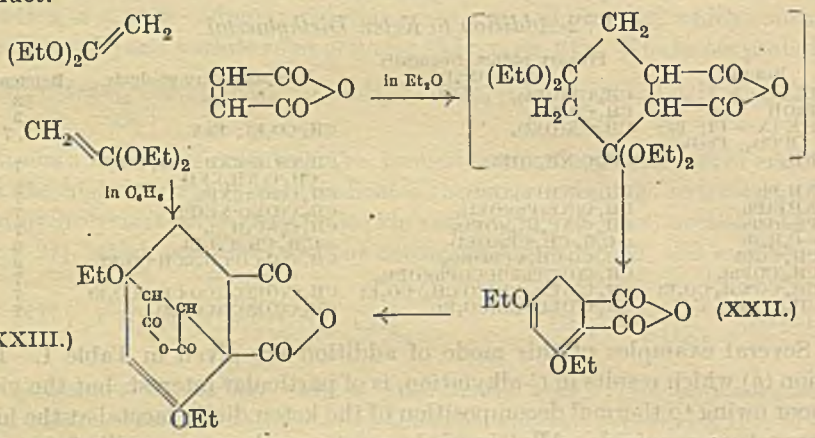
Several examples of this mode of addition are given in Table I. Reaction (*h*) which results in *C*-alkylation, is of particular interest, but the yield is poor owing to thermal decomposition of the keten diethylacetal at the high temperature required. Allyl bromide reacts much more readily,[9] but the product then contains diallylacetic ester (XIX; A = allyl). A similar result is obtained with benzyl bromide.[9] Ethyl acetoacetate is probably a secondary product from (*i*), but in the presence of the acetyl chloride it is converted into the *O*-acetyl derivative actually isolated as the main constituent; the hydrogen chloride thereby evolved attacks a further mole of keten diethylacetal, both by 1 : 4-addition (see later) and according to reaction (*c*) (X = Cl), to form ethyl acetate and ethyl chloride. Benzoyl chloride reacts in the same way.[9] In reaction (*j*), the highly enolic dibenzoylmethane adds almost quantitatively at 25°, but in (*k*) and (*l*) the yields are poor unless sodium ethoxide is present to increase the enolisation. Methylmalonic ester fails to react even under the most vigorous enolising conditions.[7] The secondary products from (*k*) and (*l*) contain ethyl orthoacetate, formed by reaction of the liberated alcohol with a further mole of keten diethylacetal.

In many instances the addition takes a different course, conveniently classified as a 1 : 4-type. This occurs when one mole of AB reacts with two moles of keten diethylacetal to give (XX); this adduct is frequently unstable and may decompose to give a number of different products.

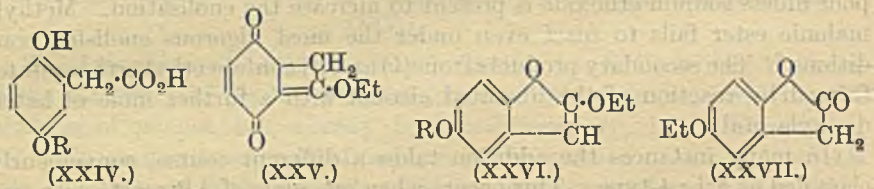
(XX.) $A{\cdot}CH_2{\cdot}C(OEt)_2{\cdot}CH_2{\cdot}CB(OEt)_2$ $CH_3{\cdot}C(OEt){:}CH{\cdot}CO_2Et$ (XXI.)

Acids of sufficient strength will bring about 1 : 4-addition, so that reaction (*c*) is frequently accompanied by the formation of ethyl β-ethoxycrotonate (XXI) derived from the primary 1 : 4-adduct by loss of EtOH and EtX.[9] The addition of bromine to bromoketen diethylacetal also follows both the 1 : 2- and the 1 : 4-route.[5] Maleic anhydride adds to keten diethylacetal exclusively in the 1 : 4-manner, and 3 : 5-diethoxy-1 : 6-di-

hydrophthalic anhydride (XXII) rapidly separates from an ethereal solution of the reactants.[10] Further reaction of maleic anhydride with the conjugated system in (XXII) may be accomplished by the use of benzene as solvent to yield (XXIII). Dimethylmaleic anhydride does not give an adduct.



p-Benzoquinone reacts only by 1 : 2-addition to give a product which on hydrolysis furnishes homogentisic acid (XXIV; R = H). The adduct was at first [10] thought to be (XXV), but subsequently [16] this formula was rejected in favour of the 5-hydroxy-2-ethoxycoumarone structure (XXVI; R = H), which accounts equally well for the production of (XXIV; R = H) on hydrolysis, but explains also the formation of 5-ethoxycoumaran-2-one (XXVII) and the corresponding acid (XXIV; R = Et) by hydrolysis of the ethyl ether of the adduct (XXVI; R = Et). Other quinones, dibenzylideneacetone, and benzylideneacetophenone also give 1 : 2-adducts with



keten diethylacetal, though the yields are generally poor.[10] With *p*-ethoxybenzenediazonium chloride, 1 : 2-addition gives the unstable intermediate (XXVIII), which decomposes and reacts with a further mole of the diazonium

p-EtO·C$_6$H$_4$·N:N·CH$_2$·CCl(OEt)$_2$ ⟶ *p*-EtO·C$_6$H$_4$·NH·N:CH·CO$_2$Et
(XXVIII.)

(XXIX.) *p*-EtO·C$_6$H$_4$·NH·N:C(CO$_2$Et)·N:N·C$_6$H$_4$·OEt-*p*

salt to give ethyl di-*p*-anisylformazylformate (XXIX). Simultaneous 1 : 4-addition, *via* the intermediate (XXX), leads to the formation of the

phenylhydrazone (XXXI) and thence 4-ethoxy-1-*p*-anisylpyridaz-6-one (XXXII). The occurrence of both types of addition appears to be general

(XXX.) p-EtO·C_6H_4·N:N·CH_2·C(OEt)$_2$·CH_2·CCl(OEt)$_2$ ⟶ (XXXI.) p-EtO·C_6H_4·NH·N:CH·C(OEt):CH·CO_2Et

↓

(XXXII.) p-EtO·C_6H_4·N<N=CH / CO—CH>C·OEt

for substituted benzenediazonium chlorides, but benzenediazonium chloride itself gives only the pyridazone, with no evidence of 1 : 2-addition.

L. N. O.

4. Aldols and Related Products.

Although it is more than seventy years since aldols were first prepared, it is only now that a clear understanding has begun to emerge of the many problems which these comparatively simple substances offer to the physical and the organic chemist. Many different opinions have been held, even within the last decade, on such aspects as the mechanism of the condensation,[1] the structure of monomeric aldols, and the changes which occur when aldols polymerise, but recent investigations have done much to clarify the position.

General.—Observations have been made on the catalysis of the aldol condensation by amino-acids [2] and by phosphorus oxychloride.[3] From a preparative point of view, these catalysts are inefficient, and with either type of reagent the aldol is usually accompanied by a considerable quantity of the corresponding αβ-unsaturated aldehyde. F. G. Fischer [4] has recorded the properties of β-methylacetaldol, which is conveniently prepared by the ozonisation of dimethylallylcarbinol, according to the method first described in 1939 by G. N. Burkhardt, I. M. Heilbron, and J. B. Aldersley.[5]

The formation of symmetrical dialkyl ketones, by the pyrolysis over chromia of normal primary alcohols, is believed [6] to involve the intermediate formation of an aldol, according to the following scheme :

$$R\cdot CH_2\cdot CH_2\cdot OH \xrightarrow{-H_2} R\cdot CH_2\cdot CHO \longrightarrow R\cdot CH_2\cdot CH(OH)\cdot CHR\cdot CHO \xrightarrow{-CO}$$
$$R\cdot CH_2\cdot CH(OH)\cdot CH_2R \xrightarrow{-H_2} R\cdot CH_2\cdot CO\cdot CH_2R$$

This mechanism is supported by the observation [7] that a higher yield of ketone is obtained when the appropriate aldol is used in place of the alcohol; *n*-butyraldol, for example, is readily converted into di-*n*-propyl ketone.

In the condensation of an aldehyde with an unsymmetrical methyl

[1] H. B. Watson, *Trans. Faraday Soc.*, 1941, **37**, 707.

[2] W. Langenbeck and G. Borth, *Ber.*, 1942, **75**, 951; E. V. Budnitskaya, *Biokhimiya*, 1941, **6**, 146.

[3] M. Backès, *Bull. Soc. chim.*, 1942, **9**, 60.

[4] *Ber.*, 1943, **76**, 734. [5] B.P. 512,465.

[6] V. I. Komarewsky and J. R. Coley, *J. Amer. Chem. Soc.*, 1941, **63**, 700.

[7] *Idem, ibid.*, p. 3269.

ketone $Me{\cdot}CO{\cdot}CH_2R$, the ketol may be formed either by reaction with the methyl group or with the α-methylene group, depending partly upon the branched or unbranched nature of the aldehyde. In an extension of some earlier work, S. G. Powell and F. Hagemann [8] have shown that *iso*butyraldehyde condenses almost exclusively with the methyl group. The structures of the ketols are established by ozonisation of the αβ-unsaturated ketones obtained on dehydration. During the course of this work, an interesting isomerisation was observed in the reduction by sodium and alcohol of 2-methyldec-3-en-5-one (I), obtained from *iso*butyraldehyde and methyl *n*-amyl ketone. The product proved to be the βγ-unsaturated alcohol (II).

(I.) $CHMe_2{\cdot}CH{:}CH{\cdot}CO{\cdot}[CH_2]_4{\cdot}Me \longrightarrow CMe_2{:}CH{\cdot}CH_2{\cdot}CH(OH){\cdot}[CH_2]_4{\cdot}Me$ (II.)

Structures of Monomeric Aldols.—Monomeric aldols may be formulated either as β-hydroxy-aldehydes (III) or as cyclic hemiacetals (IV), and the possibility of an equilibrium involving both forms has long been envisaged.[9] The open-chain structure is supported by the preparation of characteristic

(III.) $R{\cdot}CH(OH){\cdot}CH_2{\cdot}CHO \rightleftharpoons R{\cdot}CH{\cdot}CH_2{\cdot}CH{\cdot}OH$ (IV.)
$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \llcorner\!\!-O-\!\!\lrcorner$

derivatives, by reactions involving either the carbonyl or the hydroxyl function. It has not always been realised that reactions of the former type must be performed under mild conditions, and some derivatives originally ascribed to acetaldol have now been recognised as derived from crotonaldehyde.[10, 11] Reactions involving the hydroxyl group are usually restricted by the ease with which the aldols polymerise, but E. Späth and T. Meinhard [12] have succeeded in preparing pure esters of monomeric acetaldol by slow distillation of the dimeric esters under reduced pressure. As will be seen from the structure of the dimeric form (p. 142), the yield by this process cannot exceed 50% of the theoretical, but the method is nevertheless of some value.

By the aldolisation of methoxyacetaldehyde, C. D. Hurd and J. L. Abernethy [13] have prepared a 2 : 4-dimethyl aldotetrose, to which the open-chain structure (V) is assigned. The presence of the free aldehyde group is supported by a positive reaction with Schiff's reagent. They also favour an open-chain structure for acetaldol on the grounds that methylation of its dimethylacetal gives β-methoxybutyraldehyde dimethylacetal

(V.) $MeO{\cdot}CH_2{\cdot}CH(OH){\cdot}CH(OMe){\cdot}CHO \quad CH_3{\cdot}CH(OMe){\cdot}CH_2{\cdot}CH(OMe)_2$ (VI.)

(VI), but this result is inevitable, since the dimethylacetal itself can be formulated only as an open-chain derivative. The problem of structure is in fact analogous to that of the aldoses,[14] and if, as appears very probable,

[8] *J. Amer. Chem. Soc.*, 1944, **66**, 372.

[9] *Inter alia*, M. Backès, *Compt. rend.*, 1935, **200**, 1669; M. Bergmann and E. Kann, *Annalen*, 1924, **438**, 278.

[10] I. Kasuya, *J. Amer. Chem. Soc.*, 1937, **59**, 2742.

[11] E. Späth, R. Lorenz, and E. Freund, *Ber.*, 1942, **75**, 1029.

[12] *Ber.*, 1943, **76**, 504.

[13] *J. Amer. Chem. Soc.*, 1941, **63**, 1966.

[14] L. N. Owen, *Ann. Reports*, 1943, **40**, 109.

an aldol in solution is present as an equilibrium mixture of open-chain and ring forms, the isolation of derivatives of either form cannot be taken as conclusive evidence for the structure of the parent compound.

The ultra-violet absorption spectrum of acetaldol, as determined by M. Backès,[9] indicates that the presence or absence of a free carbonyl group is dependent upon the solvent used. More recently, the Raman spectra of aldols have been investigated, but it is difficult to assess the value of physical evidence in this field, particularly in view of the different interpretations assigned to such evidence by the workers concerned. For example, R. H. Saunders, M. J. Murray, F. F. Cleveland, and V. I. Komarewsky [15] found no Raman line corresponding to the frequency expected for a carbonyl group, and correctly concluded that such a group was absent.* M. Backès,[16] on the contrary, explains the unusual Raman spectrum by the postulation of a " rectified " carbonyl function, the abnormal character of which is assumed to be due to the presence of a hydroxyl group in the molecule.

The independent investigations of several schools [17, 18, 19, 20] have provided an explanation for some, at least, of the conflicting results recorded in earlier publications. It has been shown that aldols, as usually prepared in the crude state, are largely composed of an addition product with the corresponding aldehyde (see p. 144). This material may either be distilled unchanged, or may break down into one mole of aldol and one mole of aldehyde, according to the precise conditions employed. Much of the published data on aldols is therefore erroneous, and should more correctly refer to addition products of this type. Unfortunately, it is not always possible to deduce the true nature of the material examined by some workers, owing to insufficient experimental details of the method of purification.

The Polymerisation of Aldols.—Pure acetaldol, when freshly distilled under reduced pressure, is obtained as a mobile liquid which on standing becomes viscous and eventually crystallises as paraldol. On heating under reduced pressure, this substance, which has long been recognised as a dimer, is reconverted into monomeric acetaldol. Other aldols behave in a similar way. The changes which occur in this cycle have given rise to much speculation, and the earlier postulations of physical association and dissociation have more recently been discarded in favour of chemical interpretations, based on more profound variations in molecular structure.

E. Späth and H. Schmid [21] have suggested that an intermolecular condensation of the secondary hydroxyl group of one acetaldol molecule with the aldehyde group of another, to form the hemi-acetal (VII), is followed

[15] *J. Amer. Chem. Soc.*, 1943, **65**, 1309. [16] *Bull. Soc. chim.*, 1942, **9**, 274.

[17] R. H. Saunders, M. J. Murray, and F. F. Cleveland, *J. Amer. Chem. Soc.*, 1943, **65**, 1714.

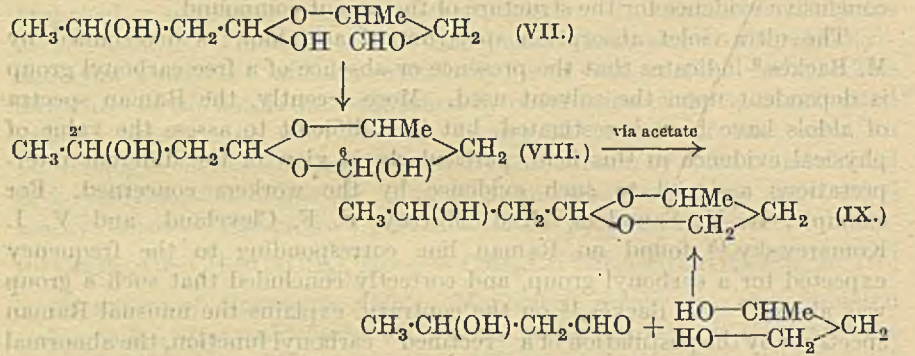
[18] *Ibid.*, 1944, **66**, 206.

[19] E. Späth, R. Lorenz, and E. Freund, *Ber.*, 1943, **76**, 57.

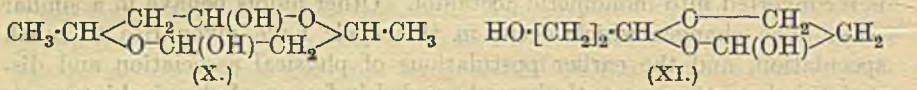
[20] E. Hanschke, *ibid.*, p. 180. [21] *Ber.*, 1941, **74**, 859.

* It was shown subsequently (refs. 17, 18) that the material under investigation was not a true aldol, but this does not invalidate the present argument.

by a similar intramolecular reaction to give paraldol, which is thus formulated as 6-hydroxy-4-methyl-2-(2′-hydroxy-*n*-propyl)-1 : 3-dioxan (VIII). This



structure is supported by the properties of paraldol diacetate, in which one acetyl residue is very readily removed by dilute mineral acid, whereas the other behaves normally; on formula (VIII), an acetyl group at position 6 would be of the hemi-acetal type, and would be expected to show great lability, in contrast with the one at position 2′. Furthermore, with hydrogen and palladium, the diacetate undergoes partial hydrogenolysis, and saponification of the product yields 4-methyl-2-(2′-hydroxy-*n*-propyl)-1 : 3-dioxan (IX), the structure of which is established by synthesis from acetaldol and 1 : 3-butanediol. The hydrogenolysis had previously been accomplished by M. Bergmann, A. Miekeley, and E. von Lippmann,[22] who correctly assigned formula (IX) to the saponified product, but suggested [23] that it was formed, by a somewhat unorthodox mechanism, from a paraldol of structure (X) * This view has been adopted, though without the production of convincing evidence, by M. Hori,[24] but it is evident that the unsymmetrical formula (VIII) accounts more readily for the difference in reactivity of the two acetyl groups, and it is also supported by the isolation of the aldol-aldehyde products described in a later section of this Report.



It is probable that 1 : 3-dioxan structures may also be allocated to the dimeric forms of other aldols.[4,25,26,27] There has been no recent investigation of dimeric hydracraldehyde, which was formulated by the Bergmann school [22,23] as a simple analogue of the eight-membered ring structure (X). It would appear very probable that it is, in fact, 6-hydroxy-2-(2′-hydroxyethyl)-1 : 3-dioxan (XI).

[22] *Ber.*, 1929, **62**, 1467. [23] M. Bergmann and A. Miekeley, *ibid.*, p. 2297.
[24] *J. Agric. Chem. Soc. Japan*, 1941, **17**, 1.
[25] E. Späth, R. Lorenz, and E. Altmann, *Ber.*, 1943, **76**, 513.
[26] E. Späth and I. von Szilágyi, *ibid.*, p. 949.
[27] E. Späth, R. Lorenz, and E. Freund, *ibid.*, p. 1196.
* (X) may theoretically be derived from (VII) by an alternative type of ring closure.

The dimerisation of acetaldol in the free state is usually complete in a few hours. This has been shown [28] by cryoscopic determinations of molecular weight, on samples of different ages after distillation, solvents such as water or dioxan being used in which the substance is relatively stable. In the presence of acetic acid the dimerisation is greatly accelerated, and the molecular weight attains a constant value, corresponding to approximately 80% of paraldol, within a few minutes.[28] On prolonged standing in aqueous solution, both acetaldol and paraldol give the same equilibrium mixture, the composition depending upon the concentration; in dilute solution there is a preponderance of monomer.[11] Substantially similar results have been obtained by M. Backès,[3] who contends, however,[29] that dimeric aldols will not yield derivatives of the monomers. This view is criticised by E. Späth, R. Lorenz, and E. Freund,[11] who have prepared several derivatives of acetaldol directly from paraldol; furthermore, as already mentioned, esters of acetaldol may be obtained by depolymerisation of the corresponding esters of paraldol.[12]

Aldol–Aldehyde Addition Products.—Assuming the validity of the mechanism outlined above for the conversion of acetaldol into paraldol, it would be anticipated that similar compounds, also with a 1 : 3-dioxan structure, would be formed by the reaction of an aldol with a simple aldehyde. This has been confirmed by E. Hanschke,[20] and by E. Späth, R. Lorenz, and E. Freund,[19] who have prepared 6-hydroxy-2 : 4-dimethyl-1 : 3-dioxan (XII), the addition product from acetaldol and acetaldehyde. A gradual scission of the product into these components is observed in aqueous solution; this may be brought about also by slow distillation under 10—15 mm., preferably in the presence of acid or alkaline catalysts, but at low pressures the substance distils without decomposition. The acetyl derivative readily undergoes hydrogenolysis to 2 : 4-dimethyl-1 : 3-dioxan (XIII), the structure of which is confirmed by synthesis from acetaldehyde

(XII.) $CH_3 \cdot CH\langle \begin{matrix} O \text{——} CHMe \\ O \text{—} CH(OH) \end{matrix} \rangle CH_2$ $\qquad$ $CH_3 \cdot CH\langle \begin{matrix} O \text{—} CHMe \\ O \text{——} CH_2 \end{matrix} \rangle CH_2$ (XIII.)

and 1 : 3-butanediol. These properties, which are strikingly similar to those of paraldol, afford strong support for the formula (VIII) assigned to the latter compound. The acetate had already been prepared by C. S. Marvel, J. Harmon, and E. H. Riddle,[30] who condensed acetaldehyde with vinyl acetate in the presence of sodium, in the hope of obtaining the acetate (XIV) of the cyclic form of acetaldol, according to the following scheme :

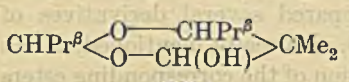
$$CH_3 \cdot CHO + CH_2{:}CH \cdot OAc \longrightarrow CH_3 \cdot \underset{\llcorner\!\text{—}O\text{—}\lrcorner}{CH} \cdot CH_2 \cdot CH \cdot OAc \quad \text{(XIV.)}$$

The properties of their product led them to suggest for it the structure assigned later to the acetate of (XII). Comparison of the physical properties of the two substances leaves no doubt that they are identical. The crystalline benzoate of (XII) [19,20] is identical with a substance, of hitherto unknown

[28] L. N. Owen, *J.*, 1943, 445.
[29] *Bull. Soc. chim.*, 1942, **9**, 69.
[30] *J. Org. Chem.*, 1939, **4**, 252.

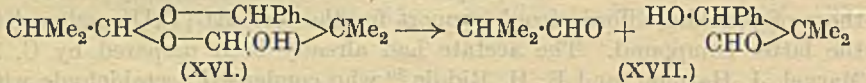
constitution, prepared in 1896 by P. C. Freer [31] from acetaldehyde, benzoyl chloride, and sodium.

The probability that crude acetaldol contains a product of structure (XII) was tentatively suggested by E. A. Shilov,[32] but its presence as a main constituent has only recently been proved by the observation that acetylation of such material gives a high proportion of the acetate of (XII). This explains a puzzling feature of many aldol condensations, in which the yield of aldol never exceeds two-thirds of the theoretical. Previously, this had been attributed to the establishment of an equilibrium between aldehyde and aldol. For example, in the aldolisation of *iso*butyraldehyde it was concluded,[33] by *distillation* of the product, that it contained 33% by weight of free aldehyde. This is precisely the yield which is obtained by the thermal decomposition of the aldol-aldehyde addition compound, 6-hydroxy-5 : 5-dimethyl-2 : 4-di-*iso*propyl-1 : 3-dioxan (XV), a substance which has been the subject of independent examinations by R. H. Saunders, M. J. Murray, and F. F. Cleveland [17] and E. Späth, R. Lorenz, and E. Freund.[27] Analogous compounds have been isolated from other crude aldols, and have also been prepared by the reaction of aldehydes with pure aldols.[18, 27] The disappearance of carbonyl groups during the progress of the reaction may be followed spectrographically.[17] It is clear that this hitherto unsuspected complication in the aldol condensation should be borne in mind when interpreting the physical data on the condensation mechanism, for which the end product has always been assumed to be the free aldol.



(XV.)

In the condensation of two or more different aldehydes, the possibilities are more numerous, and a wide range of substituted 1 : 3-dioxans is thereby made available. The crystalline Stritar product [34] prepared by the reaction of one mole of benzaldehyde and two moles of *iso*butyraldehyde is now shown [25] to be 6-hydroxy-4-phenyl-5 : 5-dimethyl-2-*iso*propyl-1 : 3-dioxan (XVI), since it may be degraded to *iso*butyraldehyde and β-hydroxy-β-phenyl-αα-dimethylpropaldehyde (XVII) :



(XVI.) (XVII.)

6-Hydroxy-5 : 5-dimethyl-2-alkyl-1 : 3-dioxans may be obtained [26] by the reaction of an appropriate aldehyde with β-hydroxy-αα-dimethylpropaldehyde (formo*iso*butyraldol).

Elimination of the hydroxyl group at position 6 can usually be accomplished by hydrogenolysis of the acetate, and hydrolysis of the products thereby formed would appear to be a useful route for the preparation of 1 : 3-glycols when the more direct method (reduction of the corresponding aldol) is not practicable.

[31] *Annalen*, 1896, **293**, 328.
[32] *J. Appl. Chem. Russia*, 1935, **8**, 93.
[33] E. H. Usherwood, *J.*, 1923, 1717.
[34] M. J. Stritar, *Monatsh.*, 1899, **20**, 617.

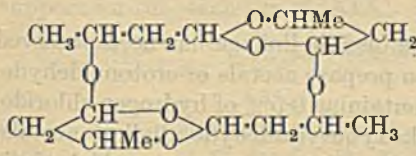
Condensations of an entirely different type have been reported by M. S. Kulpinski and F. F. Nord,[35] who have studied the catalytic effect of simple and complex alkoxides on aliphatic aldehydes. The simple alkoxides (*e.g.*, aluminium ethoxide) bring about a Cannizzaro reaction and give good yields of the simple esters; *n*-hexaldehyde, for example, gives *n*-hexyl *n*-hexoate. With complex alkoxides, such as magnesium-aluminium ethoxide, aldehydes unsubstituted in the α-position give glycol esters, which are considered to be formed by an initial aldol condensation, followed by a crossed Cannizzaro reaction between the aldol and a third mole of aldehyde :

$$R{\cdot}CH_2{\cdot}CHO \longrightarrow R{\cdot}CH_2{\cdot}CH(OH){\cdot}CHR{\cdot}CHO \longrightarrow R{\cdot}CH_2{\cdot}CH(OH){\cdot}CHR{\cdot}CH_2{\cdot}O{\cdot}CO{\cdot}CH_2R$$

If the aldehyde carries a substituent in the α-position, the yield of glycol ester is considerably reduced, and the main product is then the simple ester; this is presumably due to the less facile aldolisation of such aldehydes.

The structures assigned to the glycol esters are based mainly on the identification of their saponification products, and attention must therefore be drawn to the important observation of R. H. Saunders, M. J. Murray, and F. F. Cleveland,[17] who showed that the 1 : 3-dioxan (XV), the main product of the aldolisation of *iso*butyraldehyde, is decomposed on boiling with alcoholic alkali into *iso*butyric acid and 2 : 2 : 4-trimethylpentane-1 : 3-diol. It is suggested that the 1 : 3-dioxan is first degraded by the alkali to *iso*butyraldehyde and *iso*butyraldol (a reaction which, as mentioned earlier, is known to occur on distillation) and that these then interact, possibly *via* the glycol ester, to give the acid and the glycol. Although the refractive indices and densities of the glycol esters differ appreciably from those of the isomeric 1 : 3-dioxans, the corresponding boiling points are almost identical, and similar conclusions apply to the acetates. Further examination of the glycol esters would appear to be desirable. Their acetates would be expected to show more resistance to hydrolysis and hydrogenolysis than those of the 1 : 3-dioxans, and a direct comparison could be made with the benzoates, not yet described for the glycol esters, but known in crystalline form for some of the 1 : 3-dioxans.

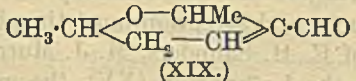
Higher Condensation Products.—In the presence of hydrogen cyanide, paraldol condenses, with loss of water, to give "tetra-aldan," $C_{16}H_{28}O_6$.[36] The product has been re-examined by E. Späth, R. Lorenz, and E. Freund,[37] who find that it contains no active hydrogen and is resistant to hydrogenation. Loss of water has therefore occurred between the hydroxyl groups present in paraldol, and the most probable structure is considered to be (XVIII).



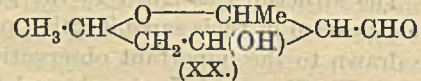
(XVIII.)

[35] *J. Org. Chem.*, 1943, **8**, 256.
[36] C. G. Lobry de Bruyn and H. C. Bijl, *Rec. Trav. chim.*, 1900, **19**, 173.
[37] *Ber.*, 1943, **76**, 722.

The substance " dialdan," obtained by the action of fuming hydrochloric acid on acetaldehyde, was considered by A. Wurtz [38] to be $C_8H_{14}O_3$. It is now shown [39] that the primary product contains an additional mole of acetaldehyde, and is $C_{10}H_{18}O_4$. It exhibits properties comparable with those of the aldol-aldehyde compounds previously considered. On boiling with water, acetaldehyde is evolved, and the free dialdan thereupon dimerises to yield a product isomeric, but not identical, with the tetra-aldan mentioned above. This " dimeric dialdan," which contains two active hydrogen atoms in the molecule, behaves in a similar way to paraldol, and when distilled at 10 mm. pressure is converted into the monomer, which again rapidly dimerises. Dehydration of the monomer yields " dimeric crotonaldehyde," the structure of which, according to M. Delépine (see p. 147) is probably (XIX), from which it follows that dialdan is (XX). On the assumption that the dimerisation of this substance is analogous to the conversion of acetaldol into paraldol, dimeric dialdan is represented by (XXI).

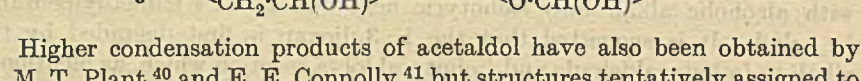


(XIX.)



(XX.)

(XXI.)



Higher condensation products of acetaldol have also been obtained by M. M. T. Plant [40] and E. E. Connolly,[41] but structures tentatively assigned to them cannot be regarded as finally established.

Crotonaldehyde and its Derivatives.—The dehydration of acetaldol to crotonaldehyde has been extensively studied for many years from the technical point of view, and improvements in the procedure are still being recorded in the patent literature.[42] The reverse process, the hydration of crotonaldehyde, is of no technical interest, but has been encountered by S. Winstein and H. J. Lucas [43] as part of a general investigation of the kinetics of the hydration of olefins. They have studied the acetaldol $\rightleftharpoons$ crotonaldehyde equilibrium in dilute aqueous mineral acid, and have determined the energies of activation of both reactions. According to W. Langenbeck and R. Sauerbier,[44] the attainment of equilibrium in aqueous solution is catalysed also by secondary amines.

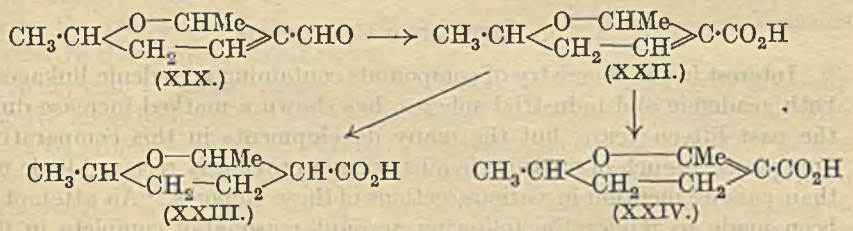
The direct addition of alcohols to the olefinic linkage has been observed by G. Meier,[45] as a result of attempts to prepare acetals of crotonaldehyde. By the use of the appropriate alcohol, containing 0·5% of hydrogen chloride, good yields are obtained of the β-alkoxybutyraldehyde dialkyl acetals. L. Rappen [46] has shown that addition also occurs when crotonaldehyde is oxidised with selenium dioxide in alcoholic solution, the product being

[38] *Compt. rend.*, 1872, **74**, 1361.
[39] E. Späth, R. Lorenz, and E. Freund, *Ber.*, 1943, **76**, 520.
[40] *J.*, 1938, 536. [41] *J.*, 1943, 42. [42] U.S.P. 2,341,229.
[43] *J. Amer. Chem. Soc.*, 1937, **59**, 1461. [44] *Ber.*, 1937, **70**, 1540.
[45] *Ber.*, 1943, **76**, 1016. [46] *J. pr. Chem.*, 1941, **157**, 177.

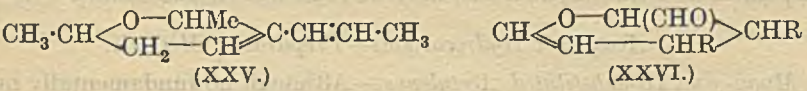
an alkoxyethylglyoxal, $CH_3{\cdot}CH(OR){\cdot}CO{\cdot}CHO$. In acetic acid solution, the corresponding acetoxy-derivative is formed.

The oxidation of crotonaldehyde in acetic acid with gaseous oxygen gives mainly crotonic acid, and although the presence of manganese is known to be advantageous under these conditions, it has now been shown [47] that the reaction is affected to a remarkable degree by the exact amount of catalyst present. At 20°, the optimum quantity of manganese is extremely small, *viz.*, 0·00001%, and there is a considerable reduction in the efficiency of oxidation if the catalyst concentration is more or less than this figure. The effect is not so marked at higher temperatures.

Linear condensation products of crotonaldehyde have recently been reviewed in these Reports,[48] but under strongly acid conditions the aldehyde yields compounds of the pyran type. "Dimeric crotonaldehyde" was formulated by M. Delépine [49] as 3-formyl-2 : 6-dimethyl-5 : 6-dihydro-1 : 2-pyran (XIX), which may be derived from the aldol of crotonaldehyde by allylic rearrangement and ring closure. In a renewed study of this material [50] it has been oxidised to the acid (XXII), which on hydrogenation in the presence of Raney nickel gives the expected dihydro-compound (XXIII) together with (XXIV), an isomer of the original acid; (XXIV) may be obtained in higher yield by performing the isomerisation with Raney nickel in the absence of hydrogen. The structure of this isomer is known with



certainty, since it is identical with an acid synthesised in 1914 by R. G. Fargher and W. H. Perkin (jun.).[51] Condensation of dimeric crotonaldehyde with ethylmagnesium iodide, and dehydration of the resulting secondary alcohol, gives the diene (XXV), the conjugated nature of which is confirmed by its ready reaction with maleic anhydride.[52] These results afford strong support for the structure of (XIX).



A new dimeric crotonaldehyde has been mentioned by K. Alder, H. Offermanns, and E. Rüden,[53] This is obtained by heating the aldehyde

[47] L. N. Owen, *J.*, 1943, 463.

[48] E. R. H. Jones, *Ann. Reports*, 1941, **38**, 170. [49] *Compt. rend.*, 1910, **150**, 535.

[50] M. Delépine and A. Horeau, *ibid.*, 1938, **206**, 27; M. Delépine and A. Willemart, *ibid.*, 1940, **211**, 153, 313.

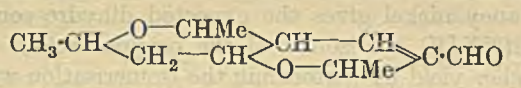
[51] *J.*, 1914, **105**, 1353.

[52] M. Delépine and P. Compagnon, *Compt. rend.*, 1941, **212**, 1017.

[53] *Ber.*, 1941, **74**, 926.

under pressure, and is formulated as 6-formyl-4 : 5-dimethyl-5 : 6-dihydro-1 : 4-pyran (XXVI; R = CH_3), on the grounds that it is probably derived by a diene condensation between two molecules of crotonaldehyde, in a manner similar to that which occurs in the dimerisation of acraldehyde under these conditions. In the latter instance, the constitution (XXVI; R = H) assigned to dimeric acraldehyde is supported by the following considerations.[54] Reduction of the dihydro-derivative by the Kishner–Wolff method gives a product which is said to be identical with 2-methyltetrahydropyran, prepared by synthesis from ethyl acetoacetate and trimethylene dibromide, though the comparison is based only on boiling point and odour. More satisfactory evidence is provided by the formation of glutaric acid on oxidation of the dihydro-derivative, and the quantitative isolation of succinic acid by oxidation of the dimer itself.

A trimeric crotonaldehyde, analogous in its properties to the dimer (XIX), has been investigated by M. Badoche,[55] who has assigned to it the structure (XXVII). With Raney nickel, the corresponding acid undergoes an isomerisation similar to that encountered in the work on the dimer.

(XXVII.) 

L. N. O.

5. Acetylenic Compounds.

Interest in the chemistry of compounds containing acetylenic linkages, in both academic and industrial spheres, has shown a marked increase during the past fifteen years, but the many developments in this comparatively unexplored branch of organic chemistry have previously received little more than passing mention in various sections of these Reports. An attempt has been made to render the following account reasonably complete in itself insofar as recent work on the hydrocarbons and alcohols is concerned, but it has not been possible to discuss the well-known reactions of acetylene of technical importance, *e.g.*, addition of halogens, halogen acids and hydration. Certain of the notable omissions have been referred to in the text and it is hoped that some of these, especially discussions of acetylenic carbonyl compounds and carboxylic acids, may be remedied in due course.

I. *Acetylenic Hydrocarbons—Preparative Methods.*

Mono- and Di-substituted Acetylenes.—Although no fundamentally novel methods have been described, such notable improvements have been made in the two classical methods, *viz.*, dehydrohalogenation and synthesis from organo-metallic derivatives of acetylenes, that these hydrocarbons are now readily available in the laboratory. In both cases advantage has been taken of the solubility and surprisingly high reactivity of alkali metal acetylides and alkali metal amides in liquid ammonia.

[54] K. Alder and E. Rüden, *Ber.*, 1941, **74**, 920.
[55] *Compt. rend.*, 1942, **214**, 845; **215**, 142; 1943, **216**, 488.

The sodamide method of dehydrohalogenation has been modified by D. Bodroux,[1] who found that the presence of a small amount of aniline (giving NHPhNa with sodamide) so facilitates the conversion of bromo-

$$CHPh{:}CHBr + NHPhNa \longrightarrow CPh{:}CH + NH_2Ph + NaBr$$

styrene into phenylacetylene (89%) and related reactions that they can be carried out in ether at the ordinary temperature. T. H. Vaughn, R. R. Vogt, and J. A. Nieuwland [2] have described a catalytic method, using sodium oxide together with ammonia-soluble iron, nickel or cobalt salts, for the preparation of sodamide, in a highly active condition, from sodium in solution in liquid ammonia. They found it to be extremely effective in dehydrohalogenating compounds such as 2-bromo-Δ^1-octene and β-bromo- and α-chloro-styrenes to the corresponding acetylenes. The use of sodamide in inert solvents at high temperatures for the same purpose suffers from the disadvantage that acetylenes frequently rearrange under these conditions, whereas no isomerisations take place with sodamide in liquid ammonia at its boiling point. When the potassium amide–liquid ammonia method is applied [3] to halogen derivatives of 1 : 1-diaryl-ethylenes and -ethanes, good

$$CAr_2{:}CHCl \longrightarrow CAr{:}CAr \longleftarrow CHAr_2{\cdot}CHCl_2$$

yields of diarylacetylenes result; this rearrangement reaction was effected earlier only by using sodium ethoxide at 180—200°. It has been noted that, during the migration of the aryl group, substituents retain their positions relative to the point of attachment.

Acetylenic Grignard reagents (≡C·MgBr) do not react with normally reactive alkyl halides, but with ethyl or methyl sulphate (2 mols.) and with esters of aromatic sulphonic acids, alkylation takes place readily.[4] These Grignard reagents will react, as is indicated in later sections of this Report, with halogens and with a variety of compounds containing activated halogen atoms, such as allyl and substituted propargyl halides, cyanogen halides, α-halogeno-ethers and acyl halides. Both alkyl halides and sulphates [5] as well as alkyl esters of aromatic sulphonic acids [6] can be employed for the preparation of a wide range of mono- and di-substituted acetylenes from solutions of the requisite sodio-derivatives in liquid ammonia. This convenient method, first employed by M. Pichon [7] for reactions with alkyl halides, has been discussed in great detail, as a result of a vast amount of experimental experience, by Vaughn, Hennion, Vogt, and Nieuwland.[5]

[1] *Compt. rend.*, 1939, **208**, 1022. [2] *J. Amer. Chem. Soc.*, 1934, **56**, 2120.

[3] G. H. Coleman and R. D. Maxwell, *ibid.*, p. 132; G. H. Coleman, W. H. Holst, and R. D. Maxwell, *ibid.*, 1936, **58**, 2310.

[4] S. D. Thorn, G. F. Hennion, and J. A. Nieuwland, *ibid.*, p. 796; J. R. Johnson, A. M. Schwartz, and T. L. Jacobs, *ibid.*, 1938, **60**, 1882.

[5] T. H. Vaughn, G. F. Hennion, R. R. Vogt, and J. A. Nieuwland, *J. Org. Chem.*, 1937, **2**, 1.

[6] A. L. Kranzfelder and F. J. Sowa, *J. Amer. Chem. Soc.*, 1937, **59**, 1490; cf. also R. Truchet, *Ann. Chim.*, 1931, **16**, 390, and J. R. Johnson *et al.*, *J. Amer. Chem. Soc.*, 1938, **60**, 1882, who employed suspensions of the sodio-derivatives in organic solvents.

[7] *Compt. rend.*, 1914, **158**, 1184, 1346; 1919, **169**, 32.

It is equally applicable to the alkylation of vinylacetylenes [8] and with β-bromo-ethers, good yields of acetylenic ethers such as (I) and (II) can be

(I.) $CH{:}C{\cdot}CH_2{\cdot}CH_2{\cdot}OR$ $CH{:}C{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}C{:}CH$ (II.)

obtained.[9] K. N. Campbell and L. T. Eby [10] prepared branched-chain acetylenes by condensing tertiary acetylenic chlorides with aliphatic Grignard reagents. The reaction is limited, however, to the halides from disubstituted acetylenes.

$$C_2H_5{\cdot}C{:}C{\cdot}CMeEtCl + EtMgBr \longrightarrow C_2H_5{\cdot}C{:}C{\cdot}CMeEt_2$$

Allylacetylenes and Diacetylenes.—During a study of the behaviour of the highly reactive halide (III), T. Y. Lai [11] found that a Wurtz type of reaction

(III.) $C_5H_{11}{\cdot}C{:}C{\cdot}CH_2Br \xrightarrow{Mg} C_5H_{11}{\cdot}C{:}C{\cdot}CH_2{\cdot}CH_2{\cdot}C{:}C{\cdot}C_5H_{11}$ (IV.)

$C_5H_{11}{\cdot}C{:}C{\cdot}CH_2{\cdot}C{:}C{\cdot}C_5H_{11}$ (V.)

with magnesium in ether readily gave the diacetylenic hydrocarbon (IV). From (III) in xylene solution, with the sodio-derivatives of acetylenes, 1 : 4-di-ynes of type (V) were obtained, the rather poor yields being attributed to the reactivity of the central methylene group, which liberated methane (1·5 mols.) from Grignard reagents (Zerewitinoff). A hydrocarbon similar to (V) has also been prepared by the action of methylene sulphate on an acetylenic Grignard reagent in the presence of cuprous chloride.[12] The work of R. Lespieau and Journaud [13] on the interaction of allyl halides with sodium acetylide in liquid ammonia provides further evidence of α-methylenic activation by an acetylenic bond. Allyl iodide gave mainly a hydrocarbon believed to be (VI) and the bromide gave (VII) in 25% yield, both (VI) and (VII) resulting from further substitution of the allylacetylene initially

(VI.) $CH{:}C{\cdot}C(CH_2{\cdot}CH{:}CH_2)_2$ $CH{:}C{\cdot}CH{\cdot}CH_2{\cdot}CH{:}CH_2$ (VII.)

$CH{:}CH_2$ $CH{:}CH_2$

formed. Reactions of acetylenic Grignard reagents with allyl bromide proceed readily in the presence of anhydrous copper halides,[14] resulting in the formation of allyl-acetylenes (*e.g.*, VIII).

The coupling of organic radicals consequent upon treatment of Grignard reagents with metallic salts has long been known and J. P. Danehy and J. A. Nieuwland [15] have shown that diacetylenes (IX) are formed in good yields

(VIII.) $C_4H_9{\cdot}C{:}C{\cdot}CH_2{\cdot}CH{:}CH_2$ $C_4H_9{\cdot}C{:}C{\cdot}C{:}C{\cdot}C_4H_9$ (IX.)

from the appropriate Grignard compounds and cupric bromide. It is suggested that the unstable organo-metallic compound first produced readily

[8] W. H. Carothers and R. A. Jacobsen, *J. Amer. Chem. Soc.*, 1933, **55**, 1622.

[9] P. A. McCusker and J. W. Kroeger, *ibid.*, 1937, **59**, 213.

[10] *Ibid.*, 1940, **62**, 1798. [11] *Bull. Soc. chim.*, 1933, **53**, 1533, 1537, 1543

[12] G. F. Hennion and E. P. Bell, *J. Amer. Chem. Soc.*, 1943, **65**, 1847.

[13] *Bull. Soc. chim.*, 1931, **49**, 423.

[14] J. P. Danehy, D. B. Killian, and J. A. Nieuwland, *J. Amer. Chem. Soc.*, 1936, **58**, 611.

[15] *Ibid.*, p. 1609.

decomposes, since with silver bromide no products other than the stable silver acetylides are obtained. The formation of diacetylenes from Grignard reagents either by aerial oxidation or by treatment with half the amount of iodine required for iodoacetylene formation had previously been observed.[16]

$$R{\cdot}C{:}C{\cdot}MgBr + I_2 \longrightarrow R{\cdot}C{:}CI \xrightarrow{R{\cdot}C{:}CMgBr} R{\cdot}C{:}C{\cdot}C{:}CR + MgBrI$$

Another classical route to diacetylenes, entailing the oxidation of copper acetylides either with air in ammoniacal solution or by means of ferricyanide, has been developed into a generally useful synthetic method by J. S. Salkind and his collaborators.[17] While attempting to apply the Nieuwland vinylacetylene synthesis ($CuCl$–NH_4Cl–HCl) to substituted acetylenes, they observed the formation of insoluble complexes (*e.g.*, $C_{16}H_{10}CuCl$ from $Ph{\cdot}C{:}CH$), which decomposed in contact with ether, giving diacetylenes. The reaction proceeds with the absorption of the theoretical amount of atmospheric oxygen and the presence of mineral acids is not necessary; it gives high yields of glycols (X) when tertiary acetylenylcarbinols are employed in place of the hydrocarbons.

(X.) $CMe_2(OH){\cdot}C{:}C{\cdot}C{:}C{\cdot}C(OH)Me_2$

Vinylacetylenes.—The discovery [18] of a practicable method for the catalytic polymerisation of acetylene has led not only to the notable technical achievement of the production of a synthetic rubber from polymerised chloroprene but has also focused attention on the chemistry of the conjugated vinylacetylenes. The production of vinylacetylene itself is accompanied by further polymerisation, including trimerisation to *s.*- (XI) and *as.*-divinylacetylenes (XII) (ethynylbutadiene).[18, 19] These isomers can be

(XI.) $CH_2{:}CH{\cdot}C{:}C{\cdot}CH{:}CH_2$ $\qquad$ $CH_2{:}CH{\cdot}CH{:}CH{\cdot}C{:}CH$ (XII.)

separated either by making use of the reaction of (XII) with ammoniacal cuprous chloride, distilling off the unreacted isomer, and regenerating (XII) with acids, or simply by hydrating the more reactive (XII) with acidified mercuric salts.[20]

The application of the elegant α-olefin synthesis of H. B. Dykstra, J. F. Lewis, and C. E. Boord [21] to a mixture of acetylene mono- and di-magnesium bromides gives vinyl- and divinyl-acetylenes. With 1 : 2-dibromoethyl

[16] V. Grignard and Tschéoufaki, *Compt. rend.*, 1929, **188**, 357; V. Grignard and H. Perrichon, *Ann. Chim.*, 1926, **5**, 14.

[17] *Ber.*, 1936, **69**, 128; *J. Gen. Chem. Russia*, 1937, **7**, 227; 1939, **9**, 1725.

[18] J. A. Nieuwland, W. S. Calcott, F. B. Downing, and A. S. Carter, *J. Amer. Chem. Soc.*, 1931, **53**, 4197.

[19] U.S.P. 1,811,959; H. Schmitz and H. J. Schumacher, *Z. Elektrochem.*, 1939, **45**, 503; P. W. Shaworonkow, A. P. Allechina, and R. J. Ster, *Chem. Zentr.*, 1935, I, 1625; A. L. Klebanski, L. G. Zurich, and I. M. Dolgopolski, *Bull. Acad. Sci. U.R.S.S.*, 1935, **7**, 189; A. L. Klebanski, U. A. Dranitzina, and I. M. Dobromilskaja, *Chem. Zentr.*, 1936, I, 2528.

[20] A. S. Carter and du Pont, U.S.PP. 2,263,378, 2,228,752, 2,173,272; see also Klebanski *et al.*, *Chem. Zentr.*, 1936, I, 2528.

[21] *J. Amer. Chem. Soc.*, 1930, **52**, 3396.

ethyl ether the Grignard reagents give the bromo-ethers (XIII and XIV) and, on reduction with zinc and an alcohol, the two vinylacetylenes are

$CH{:}C{\cdot}CH(OEt){\cdot}CH_2Br$ (XIII.) $\qquad$ $CH_2Br{\cdot}CH(OEt){\cdot}C{:}C{\cdot}CH(OEt){\cdot}CH_2Br$ (XIV.)

formed.[22] This method has been examined further [23] and extended to the preparation of substituted vinylacetylenes ($R{\cdot}C{:}C{\cdot}CH{:}CHR'$), and provides a useful adjunct to the more restricted alkylation of sodio-vinylacetylenes in liquid ammonia.[8]

Probably the most convenient route to the conjugated vinylacetylene system, however, is that originated by G. Merling.[24] It takes advantage of the comparatively facile dehydration of the *tert.*-acetylenylcarbinols, derived from condensations between ketones and acetylenic hydrocarbons, *e.g.*,

$$CH{:}C{\cdot}C(OH)Me_2 \xrightarrow{Ac_2O\text{-}H_2SO_4} CH{:}C{\cdot}CMe{:}CH_2$$

A considerable variety of dehydrating agents has been employed and among the most successful are acetic anhydride containing a little sulphuric acid,[25] and magnesium sulphate [24, 26] or alumina [25, 27] used for vapour-phase dehydrations at about 250°. Strongly acidic reagents such as formic, sulphuric, phosphoric, and toluene-*p*-sulphonic acids and potassium hydrogen sulphate are quite effective in particular cases, but their general use is restricted on account of the rearrangements or hydrations which accompany, or occur to the exclusion of, dehydration. Strongly alkaline agents cannot be employed, since they bring about fission (see p. 169).

Substituted divinylacetylenes are analogously produced by dehydration

$$CMe_2(OH){\cdot}C{:}C{\cdot}C(OH)Me_2 \xrightarrow[H_3PO_4]{60\%} CH_2{:}CMe{\cdot}C{:}C{\cdot}CMe{:}CH_2$$

of the di-*tert.*-acetylenic glycols or the vinylacetylenylcarbinols, and sulphuric,[28] phosphoric,[29] and toluene-*p*-sulphonic [30] acids, acetic anhydride and sulphuric acid,[31] and potassium hydrogen sulphate [32] have all been utilised successfully as dehydrating agents.

Acetylenic Hydrocarbons—Reactions.

Isomerisation.—The rearrangement of disubstituted Δ^2- and Δ^3-acetylenes to monosubstituted acetylenes [33] has been considerably extended by T. H.

[22] R. Lespieau, *Compt. rend.*, 1932, **195**, 245.

[23] W. F. Anzilotti and R. R. Vogt, *J. Amer. Chem. Soc.*, 1939, **61**, 572.

[24] Bayer and Co., D.R.-P. 290,558; Friedländer, 1914—16, **12**, 61.

[25] A. F. Thompson, J. G. Burr, and E. N. Shaw, *J. Amer. Chem. Soc.*, 1941, **63**, 186.

[26] A. I. Zacharova, *Chem. Zentr.*, 1938, II, 4216.

[27] A. F. Thompson and C. Margnetti, *J. Amer. Chem. Soc.*, 1942, **64**, 573.

[28] R. P. Linstead and A. L. Walpole, *J.*, 1939, 842.

[29] D. T. Mitchell and C. S. Marvel, *J. Amer. Chem. Soc.*, 1933, **55**, 4276; J. S. Salkind and S. W. Smagina, *J. Gen. Chem. Russia*, 1937, **7**, 470.

[30] A. T. Babajan, *ibid.*, 1939, **9**, 1410.

[31] I. N. Nazarov, *Bull. Acad. Sci. U.R.S.S.*, 1938, 695.

[32] C. S. Marvel *et al.*, *J. Amer. Chem. Soc.*, 1937, **59**, 2662, 2666, 2669; 1939, **61**, 2003; 1940, **62**, 2659.

[33] *Inter al.*, H. H. Guest, *ibid.*, 1928, **50**, 1744; M. Bourguel, *Ann. Chim.*, 1925, **3**, 205.

Vaughn,[34] who found that the Δ^6-acetylene (I), heated with sodamide, gave a 28% yield of 1-dodecyne (II), *via* its sodio-derivative. It has been shown by C. D. Hurd and R. E. Christ [35] that, although the purely thermal

$$\text{(I.)}\quad C_5H_{11}\cdot C{:}C\cdot C_5H_{11} \xrightarrow[\text{paraffin at } 210^\circ]{\text{NaNH}_2 \text{ in}} C_{10}H_{21}\cdot C{:}CH \quad \text{(II.)}$$

isomerisation of 1-heptyne and 1-hexyne gives some of the isomeric 1 : 2-allenes, it requires such high temperatures (500—600°) that extensive cracking occurs simultaneously. No 1 : 3-dienes or 2-alkynes could be detected in the products formed under these conditions.

An intensive study of the behaviour and interconversions of acetylenes, allenes, and conjugated dienes over heated silicates (floridin) at temperatures ranging from 275° to 325° has been made by J. M. Slobodin.[36] Mono-substituted acetylenes are partially converted into 1 : 2-allenes under these conditions, an equilibrium sometimes being established; propyne gives a mixture containing 40% of allene, and 1-butyne gives methylallene. The allene formed may, if the structure permits, isomerise further to the 1 : 3-diene and in one case, the reversibility of this reaction has been established,

$$CH_2{:}CMe\cdot CH{:}CMe_2 \rightleftharpoons CMe_2{:}C{:}CMe_2 \quad \text{(III.)}$$

the equilibrium mixture consisting mainly of the allene (III). In this connexion a patent claim [37] for the conversion (to the extent of 12%) of 1-butyne into butadiene over activated alumina at 300—400° is of interest. At 290° over floridin the following equilibria, largely in favour of the phenyl-

$$\text{(IV.)}\quad CH{:}C\cdot CH_2\cdot CH_2Ph \rightleftharpoons CH_2{:}C{:}C\cdot CH_2Ph \rightleftharpoons CH_2{:}CH\cdot CH{:}CHPh$$

butyne (IV), are said to be established.[38] R. J. Levina and E. M. Panov [39] mention that over chromium sesquioxide, (IV) gives some 30% of an isomer believed to be 1-phenyl-1-butyne.

Some extremely facile isomerisations and disproportionations of both acetylenic and ethylenic *cyclo*hexane derivatives over platinised charcoal (25%) at 200° have been reported.[40] Ethynyl*cyclo*hexane (V) yields a mixture of ethylbenzene (65%) and ethyl*cyclo*hexane (35%), ethynyl-Δ^1-

$$\text{(V.)}\quad 3C_6H_{11}\cdot C{:}CH \longrightarrow 2C_6H_5Et + C_6H_{11}Et$$

*cyclo*hexene is isomerised entirely into ethylbenzene, and from 4-*cyclo*hexyl-1-pentyne a 2 : 1-mixture of amylbenzene and amyl*cyclo*hexane is obtained, the ratio being reversed when the corresponding 1-pentene is employed. The distance of the acetylenic linkage from the nucleus has no adverse effect on the ease of conversion and, instead of a complete dehydrogenation–hydrogenation mechanism, Levina visualises the progression of the

[34] *J. Amer. Chem. Soc.*, 1933, **55**, 3453. [35] *Ibid.*, 1937, **59**, 2161.

[36] J. M. Slobodin, *J. Gen. Chem. Russia*, 1934, **4**, 778; 1935, **5**, 48; 1936, **6**, 1806, 1892; 1937, **7**, 1664; 1938, **8**, 1220.

[37] U.S.P. 2,325,398. [38] J. M. Slobodin, *J. Gen. Chem. Russia*, 1939, **9**, 272.

[39] *Ibid.*, 1941, **11**, 527.

[40] R. J. Levina *et al.*, *ibid.*, 1937, **7**, 353, 1866; 1938, **8**, 1776; 1939, **9**, 825; 1942, **12**, 422.

unsaturation towards the nucleus through successive acetylene ⟶ allene ⟶ diene rearrangements. This hypothesis becomes more convincing

(VI.) $C_6H_{11}\cdot CMe_2\cdot CH_2\cdot CH_2\cdot CMe{:}CH_2$ $C_6H_{11}\cdot CH_2\cdot CH_2\cdot CH_2\cdot CMe{:}CH_2$ (VII).

by the fact that the hydrocarbon (VI) remains unaffected under the conditions usually employed, whereas (VII) reacts readily.

Following the syntheses [41] of 15- and 17-membered cyclic acetylenes from the corresponding olefins, attempts have been made to determine the limiting size of the ring that can accommodate an acetylenic linkage. By dehydrohalogenation of a halogenated *cyclo*pentene only a *cyclo*pentadiene was obtained and from an analogous *cyclo*heptene derivative, a *cyclo*heptadiene containing an allene system (according to ozonolysis) was isolated.[42] N. A. Domnin,[43] however, proceeding from *cyclo*octanone *via* 1-chloro-Δ^1-*cyclo*octene, succeeded in preparing the simplest cyclic acetylenic hydrocarbon *cyclo*octyne, giving suberic acid on oxidation.

Oxidation.—Ozonolysis of the triple bond generally gives rise directly to carboxylic acids,[44] although the yields are usually low, but with certain disubstituted acetylenes small quantities of α-diketones (*e.g.*, benzil and $Ph\cdot CO\cdot CO\cdot CH_2Ph$) can be isolated.[45] W. N. Krestniski and M. K. Kelbovskaja [46] conducted careful oxidations of acetylenic hydrocarbons with permanganate (equivalent to one atom of oxygen) and, failing to detect any neutral products, concluded that the oxidative behaviour of the triple bond is entirely different from that of the ethenoid linkage. It has been stated [47] that, although acetylenic bonds are slowly oxidised with peracetic acid, mainly to acidic products, the course of the oxidation is more complicated than that of ethenoid linkages.

The formation of unstable peroxides when acetylenic hydrocarbons are kept in contact with air or oxygen has been definitely established [48] and with oxygen, at 40° over a period of 3 months, 1-hexyne was partially converted into valeric acid. M. J. Murray and F. F. Cleveland [49] found that diamylacetylene (VIII) and similar hydrocarbons underwent aerial oxidation

(VIII.) $C_5H_{11}\cdot C{:}C\cdot CH_2\cdot C_4H_9 \xrightarrow{air} C_5H_{11}\cdot C{:}C\cdot CO\cdot C_4H_9$

to αβ-acetylenic ketones. α-Methylenic oxidation has also been observed with selenium dioxide, 1-heptyne, 1-octyne, and 1-phenyl-1-butyne all being transformed into secondary acetylenylcarbinols.[50]

[41] L. Ruzicka, M. Hurbin, and H. A. Boekenoogen, *Helv. Chim. Acta*, 1933, **16**, 498.

[42] A. E. Favorski, *J. Gen. Chem. Russia*, 1936, **6**, 720; N. A. Domnin, *ibid.*, 1939, **9**, 1983.

[43] *Ibid.*, 1938, **8**, 851.

[44] C. D. Hurd and R. E. Christ, *J. Org. Chem.*, 1936, **1**, 141; H. Paillard and C. Wieland, *Helv. Chim. Acta*, 1938, **21**, 1356.

[45] T. L. Jacobs, *J. Amer. Chem. Soc.*, 1936, **58**, 2272.

[46] *Ber.*, 1935, **68**, 512.

[47] J. Böeseken and G. Sloof, *Rec. Trav. chim.*, 1930, **49**, 95.

[48] C. A. Young, R. R. Vogt, and J. A. Nieuwland, *J.*, 1935, 115; *J. Amer. Chem. Soc.*, 1936, **58**, 55.

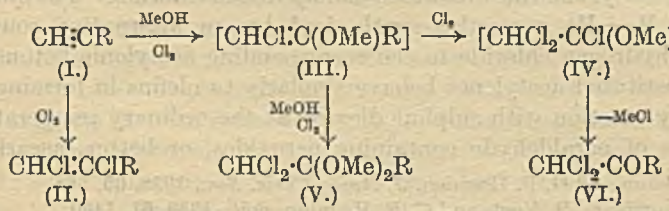
[49] *Ibid.*, 1941, **63**, 1363. [50] R. Truchet, *Compt. rend.*, 1933, **196**, 706, 1613.

Addition Reactions.—An account of the mercuric oxide–boron trifluoride catalysed additions of alcohols and carboxylic acids to acetylenes has appeared recently in these Reports.[51] Any discussion of the semihydrogenation of the acetylenic bond has had to be deferred because of limitations of space, but this aspect of addition reactions has been reviewed by K. N. Campbell and B. K. Campbell.[52]

Halogen acids and other adducts add to acetylenic linkages according to Markownikoff's rule, but with hydrogen bromide, a "peroxide effect" is definitely observed. The "normal" reaction with 1-hexyne, leading to 2-bromo-1-hexene, is extremely slow [53] and a catalyst has to be employed to effect addition of hydrogen chloride (see below). The addition of hydrogen fluoride both to acetylene and to its homologues has been studied.[54] At low temperatures good yields of the almost completely inert difluoroparaffins are obtained, the 1-alkynes, for example, giving 2 : 2-difluoro-compounds according to the Markownikoff rule. Only with acetylene itself was any of the intermediate ethylenic monofluoride isolated.

Although the literature on the addition of halogens and halogen acids to acetylene is extensive, additions to substituted acetylenes have been neglected until recently. Working in inert solvents, G. F. Hennion and C. E. Welsh [55] found that the presence of a little antimony pentachloride was necessary to ensure the smooth addition of chlorine to 1-hexyne (I); then, as was expected, a mixture consisting solely of 1 : 2-dichloro-1-hexene (II) (the *trans*-form) and the tetrachloride was obtained. Reaction with hydrogen chloride also could only be effected in the presence of a catalyst, giving, with bismuth chloride, both 2-chloro-1-hexene and 2 : 2-dichlorohexane. The formation of only *cis*-1 : 2-dichloro-1-hexene (II), together with the tetrachloride, on chlorination of 2-chloro-1-hexene revealed a peculiar disproportionation.

Halogenation in reactive solvents, on the other hand, gave rise to a greater variety of products. From the complex mixture resulting from chlorination of 1-hexyne in methyl alcohol, in addition to the *trans*-form of the dichlorohexene (II), the ketone (VI) and the dichloro-ketal (V) were isolated.[56] The formation of the intermediate (III) is regarded as taking



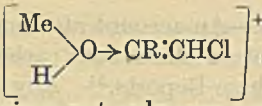
[51] F. S. Spring, *Ann. Reports*, 1942, **39**, 128. [52] *Chem. Reviews*, 1942, **31**, 77.

[53] C. A. Young, R. R. Vogt, and J. A. Nieuwland, *J. Amer. Chem. Soc.*, 1936, **58**, 1806; see also F. R. Mayo and C. Walling, *Chem. Reviews*, 1940, **27**, 351.

[54] A. L. Henne and E. P. Plueddeman, *J. Amer. Chem. Soc.*, 1943, **65**, 587; A. V. Grosse and C. B. Linn, *ibid.*, 1942, **64**, 2289.

[55] *Ibid.*, 1940, **62**, 1367.

[56] J. J. Verbanc and G. F. Hennion, *ibid.*, 1938, **60**, 1711.

place through the positive ion  , and the scheme indicated above seems reasonable, since not only was methyl chloride actually isolated, but the spontaneous decomposition of compounds such as (IV) had been demonstrated earlier.[57] Further, the ethylenic ether (III), prepared independently by the elimination of methyl alcohol from the requisite chloro-ketal, was shown to be converted into (V) on chlorination in methyl alcohol. In an aqueous medium the chlorination products, found in approximately equal amounts, were the *trans*-dichloride (II), 1 : 1 : 2-trichloro-1-hexene, the tetrachloride, and the ketone (VI), but in *tert.*-butyl alcohol the product consisted almost entirely of the *trans*-dichloride (II). When either acetic acid or its anhydride was used as solvent, the main product was the ketone (VI). In these cases, only the *cis*-form of (II) was

$$CHCl_2{\cdot}C(OAc)ClR \longrightarrow CHCl_2{\cdot}COR\ (VI.) + AcCl$$

present, and the isolation of acetyl chloride suggests that ketone formation results from the fission indicated above.[58] In the presence of mineral acids the *cis*- and the *trans*-form of 1 : 2-dichloro-1-hexene (II) were produced simultaneously; the factors governing the production of either or both of these isomers are as yet obscure.[59]

By a Friedel–Crafts reaction with aluminium chloride and acetyl chloride, acetylene yields the somewhat unstable β-chlorovinyl ketone (I), which is believed to possess the *cis*-configuration.[60] The more general reaction with

$$CH{:}CH + Me{\cdot}COCl \xrightarrow{AlCl_3} Me{\cdot}CO{\cdot}CH{:}CHCl \quad (I.)$$

(II.) $Me{\cdot}CO{\cdot}CR{:}CR'Cl$ $\qquad$ $R{\cdot}CCl{:}CHR'$ (III.)

both mono- and di-substituted acetylenes is best effected,[61] although the yields are poor, by using stannic chloride as condensing agent, and leads to a mixture of the interconvertible *cis*- and *trans*-forms of the ketones (II), together with some of the hydrogen chloride adducts (III). It is suggested that the formation of both (II) and (III) can be explained by the initial fission of the acetyl chloride into keten and hydrogen chloride. The ethylenic ketones (II, R = H) were also synthesised by an alternative route, by addition of hydrogen chloride to the corresponding acetylenic ketones.

Monosubstituted acetylenes behave similarly to olefins in forming polysulphones by reaction with sulphur dioxide at the ordinary temperature in the presence of paraldehyde containing peroxides, or better, ascaridole.[62]

[57] A. A. Baum and G. F. Hennion, *J. Amer. Chem. Soc.*, 1938, **60**, 568.

[58] R. O. Norris, R. R. Vogt, and G. F. Hennion, *ibid.*, 1939, **61**, 1460.

[59] R. O. Norris and G. F. Hennion, *ibid.*, 1940, **62**, 449.

[60] A. Cornillot and R. Alquier, *Compt. rend.*, 1935, **201**, 837.

[61] J. W. Kroeger, F. J. Sowa, and J. A. Nieuwland, *J. Org. Chem.*, 1936, **1**, 163.

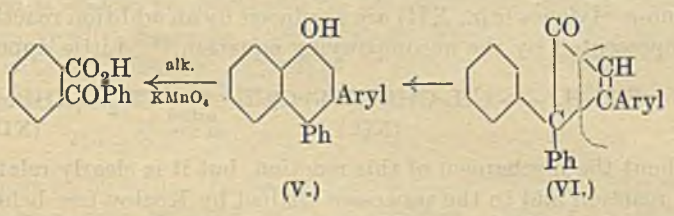
[62] L. L. Ryden and C. S. Marvel, *J. Amer. Chem. Soc.*, 1935, **57**, 2311; 1936, **58**, 2047; L. L. Ryden, F. T. Glavis, and C. S. Marvel, *ibid.*, 1937, **59**, 1014; C. S. Marvel and W. W. Williams, *ibid.*, 1939, **61**, 2710, 2714. For a general survey of the reaction with both olefins and acetylenes, see R. D. Snow and F. E. Frey, *Ind. Eng. Chem.*, 1938, **30**, 176.

Mixtures of olefins and monosubstituted acetylenes will copolymerise with sulphur dioxide, but polymerisation with disubstituted acetylenes or those of the type $CH{:}C{\cdot}CHR_1R_2$ has not been achieved. The acetylene–sulphur dioxide polymers, which have the composition $(C_nH_{2n-2}SO_2)_x$, are amorphous solids of moderately high melting points [*e.g.* $(C_6H_{10}SO_2)_n$ has m. p. 160—169°], which in fibre form show unusually well defined *X*-ray diffraction patterns. They lose half of their sulphur dioxide on heating, are hydrolysed by 10% alkali, and on oxidation with permanganate give acids containing one carbon atom less than the original hydrocarbon. By analogy with the structures established for the olefin polymers, the formulation (IV) has been proposed, but no further information concerning either the relative orient-

$$\text{(IV.)}\quad (\cdot CR{:}CH{\cdot}SO_2\cdot)_n \xrightarrow{KMnO_4} R{\cdot}CO_2H$$

ation of adjacent hydrocarbon units or the nature of the terminal groups has been obtained. Pyrolysis of the polysulphone from 1-pentyne yields an αβ-unsaturated sulphone ($C_{10}H_{16}SO_2$) which might be a di-*n*-propylthiophen-sulphone. Since the pyrolysis product could not be completely hydrogenated, however, comparisons with synthetic 2 : 5- and 3 : 4-di-*n*-propyltetrahydrothiophen sulphones could not be made.

Contrary to an earlier statement in the literature, L. I. Smith and H. H. Hoehn [63] found that arylacetylenes (Ar·C:CH) and diphenylketen interact at the ordinary temperature, in the absence of solvents, giving good yields of



(V.) (VI.)

3-aryl-4-phenyl-α-naphthols (V), their structures being substantiated by oxidative degradation and independent synthesis. The presence of solvents exerts a very definite retarding effect on the reaction and explains earlier failures. In a similar manner diphenylacetylene yields 2 : 3 : 4-triphenyl-α-naphthol, although the addition reaction is not so facile and has to be effected at 70—80°. The mechanism of this reaction has yet to be ascertained; Smith suggests that the *cyclo*butenone (VI) is the fundamental intermediate, but attempts to provide proof of this hypothesis, by synthesising a (VI) from 2 : 2 : 3-triphenyl*cyclo*butanone by dehydrogenation, were unsuccessful. Normal 1 : 2-addition of phenylacetylenylmagnesium bromide to diphenylketen takes place in boiling ethereal solution, giving the acetylenic ketone

$$Ph{\cdot}C{:}CMgBr + O{:}C{:}CPh_2 \longrightarrow Ph{\cdot}C{:}C{\cdot}CO{\cdot}CHPh_2 \quad \text{(VII.)}$$

(VII), readily hydrogenated to the saturated ketone, which was synthesised by an alternative route. Attempts to convert (VII) into the diphenyl-α-

[63] *J. Amer. Chem. Soc.*, 1939, **61**, 2619; 1941, **63**, 1175.

naphthol (V) were unavailing and it is unlikely that the acetylenic ketone is an intermediate in the formation of (V).

The preparation and reactions of amino-compounds derived from acetylenic hydrocarbons by either addition or substitution processes have received some attention within recent years, especially in the patent literature. The Mannich reaction [64] applied to phenylacetylene gives acetylenic amines of the type (VIII), which on hydration with moderately concentrated sulphuric acid yield aminopropiophenones ($Ph{\cdot}CO{\cdot}CH_2{\cdot}CH_2{\cdot}NR_2$).[65] This

$$Ph{\cdot}C{:}CH + H{\cdot}CHO + NHR_2 \xrightarrow[100°]{\text{dioxan}} Ph{\cdot}C{:}C{\cdot}CH_2{\cdot}NR_2 \quad \text{(VIII.)}$$

reaction has been extended to the vinylacetylene series,[66] where good yields of the amines (IX) can be obtained. Both mono- and di-amines of the acetylenic series are produced from acetylene itself. Thus, reaction of

(IX.) $CH_2{:}CH{\cdot}C{:}C{\cdot}CH_2{\cdot}NBu_2$ $\qquad$ $CH{:}C{\cdot}CH_2{\cdot}NBu_2$ (X.)

$NBu_2{\cdot}CH_2{\cdot}C{:}C{\cdot}CH_2{\cdot}NBu_2$ (XI.)

dibutylamine with formaldehyde in slightly acid solution in the presence of cuprous chloride, followed by treatment with acetylene under pressure at 30—40°, yields 80% of (X) and 10% of the diamine (XI).[67]

When acetylene diluted with nitrogen under pressure reacts with ammonia or a primary or secondary amine at about 60° in the presence of copper or a similar acetylide and sufficient aliphatic acid (*e.g.*, acetic) to form the amine salt, amino-acetylenes (*e.g.*, XII) are produced by an addition reaction which can be represented by the accompanying equation.[68] Little appears to be

$$2CH{:}CH + Ph{\cdot}NH_2 \longrightarrow \underset{\text{(XII.)}}{CH_3{\cdot}CH(NHPh){\cdot}C{:}CH} \xrightarrow[\text{at 250°}]{\text{Al + Fe oxides}} \underset{\text{(XIII.)}}{CH_2{:}C(NHPh){\cdot}CH{:}CH_2}$$

known about the mechanism of this reaction, but it is clearly related to the Mannich reaction and to the processes studied by Kozlov (see below). The amino-acetylenes (XII) are isomerised almost quantitatively into butadiene derivatives (XIII) when passed over mixed oxides of aluminium and iron at 250°.[69]

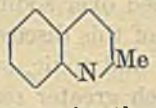
Similar 2-aminobutadienes, presumably of the type $CH_2{:}C(NR_2){\cdot}CH{:}CH_2$, are said to be produced [70] by the addition of secondary amines to vinylacetylene in the presence of cuprous chloride, the reaction being carried out in toluene solution in an autoclave at 105°.

The condensation of acetylene with primary aromatic amines in the presence of copper and other salts gives quinaldines (XVI), together with some of the tetrahydro-derivatives, and many examples of this process, leading to a variety of substituted quinaldines, have been provided by

[64] F. F. Blicke, *Organic Reactions*, 1942, **1**, 303.
[65] C. Mannich and F. T. Chang, *Ber.*, 1933, **66**, 418.
[66] D. D. Coffmann, *J. Amer. Chem. Soc.*, 1935, **57**, 1978.
[67] I. G. Farbenind., B.P. 510,904. [68] *Idem.*, B.P. 510,457; U.S.P. 2,342,493.
[69] W. Reppe *et al.*, U.S.P. 2,301,971.
[70] I. G. Farbenind., D.R.-P. 731,559; *Chem. Zentr.*, 1943, II, 1233.

N. S. Kozlov and his collaborators [71] in the course of an extensive investigation. The reaction is obviously closely allied to the classical Döbner–von Miller synthesis and, as might be expected, it proceeds by a similar mechanism, *i.e.*, *via* mono- (XIV) and di-ethylidene (XV) bases, which have both been isolated in a number of cases, the latter in both *cis*- and *trans*-forms.

(XIV.) $Ph\cdot N{:}CHMe \longrightarrow Ph\cdot NH\cdot CHMe\cdot CH{:}CH\cdot NHPh$ (XV.)

$\longrightarrow$  (XVI.) + tetrahydro-(XVI) + $PhNH_2$

The first stage must comprise the direct addition of acetylene to the base, giving a vinylamine which then isomerises to (XIV), since hydration to acetaldehyde is impossible under the anhydrous conditions involved.

Substitution Halogenation.—The replacement halogenation of acetylenic hydrogen atoms, which can be effected in a variety of ways, is always more facile in compounds ($R\cdot C{:}CH$) where the acetylenic bond is conjugated with either a benzenoid nucleus or an ethylenic linkage (*e.g.*, R = Ph or $\cdot CH{:}CH\cdot$). One of the most general and convenient methods consists in treatment with sodium hypochlorite or hypobromite in a strongly alkaline medium,[72] but as the hypochlorite reaction is ten times slower than that with hypobromite,

$$R\cdot C{:}CH + OX' \longrightarrow R\cdot C{:}CX + OH'$$

the method is said to be unsuitable for the chlorination of alkylacetylenes. It has been applied, however, both to diacetylene and to ethynylcarbinols. Another widely applicable procedure is that of R. Truchet [73] whereby halogenoacetylenes, including the chloro-derivatives of the alkylacetylenes,

$$R\cdot C{:}CNa + Ar\cdot SO_2X \longrightarrow R\cdot C{:}CX + Ar\cdot SO_2Na$$

are obtained in 50—70% yields by interaction of ethereal suspensions of sodioacetylenes with aromatic sulphonyl halides. Similar reactions ensue, but poorer yields result, with the Grignard reagents. Both the latter in ethereal solutions and the corresponding sodio-derivatives in solution in liquid ammonia give iodo-acetylenes simply by treatment with iodine,[16, 34, 74] but direct replacement by the other halogens can only be accomplished effectively at very low temperatures,[75] where addition and polymerisation processes are reduced to a minimum. A useful alternative method of obtaining bromo-acetylenes (60—80% yields) is by the interaction of acetylenic

$$Ph\cdot C{:}CMgBr + CNBr \longrightarrow Ph\cdot C{:}CBr + MgBrCN$$
$$C_5H_{11}\cdot C{:}CMgBr + CNCl \longrightarrow C_5H_{11}\cdot C{:}C\cdot CN + MgBrCl$$

[71] *J. Gen. Chem. Russia*, 1936, **6**, 250, 1089, 1341, 1346, 1897; 1937, **7**, 51, 832, 1082, 1861, 2301; 1938, **8**, 413, 419.

[72] F. Strauss, L. Kollek, *et al.*, *Ber.*, 1930, **63**, 1868, 1886.

[73] *Ann. Chim.*, 1931, **16**, 309.

[74] T. H. Vaughn and J. A. Nieuwland, *J. Amer. Chem. Soc.*, 1932, **54**, 787; 1934, **56**, 1207; 1933, **55**, 2150.

[75] P. A. McCusker and R. R. Vogt, *ibid.*, 1937, **59**, 1307.

Grignard reagents with cyanogen bromide. The use of cyanogen chloride, on the other hand, leads to good yields of acetylenic nitriles.[76]

Dichloroacetylene (C_2Cl_2) is produced by the action of hypochlorite on acetylene,[72] but the most convenient and safe method of preparation is that of E. Ott and his associates,[77] who, by passing a mixture of trichloroethylene and ether over alkalis at 130°, obtain a stable molecular compound (1 : 1; b. p. 32·5°) with ether, which can be dried over sodium and kept for long periods at − 1°. By taking advantage of this discovery, a detailed study of this interesting, but highly explosive and toxic, substance has become possible.[78] It is characterised by a much greater reactivity than that of the monochloroacetylenes, which incidentally are obtained from it by the action of Grignard reagents. Addition reactions take place with considerable

$$CCl{:}CCl + RMgX \longrightarrow R{\cdot}C{:}CCl + MgXCl$$

facility, it polymerises much more readily than acetylene, heating or irradiating an ethereal solution sufficing to produce much hexachlorobenzene. With ammonia and both primary and secondary amines the initial product in all cases appears to result from addition to the triple bond. Diethylamine addition yields the unstable dichlorovinylamine (I), readily hydrolysed to *N*-diethylchloroacetamide, and with an excess of the amine, (II) is formed.

$$CCl{:}CCl \xrightarrow{NHEt_2} CHCl{:}CCl{\cdot}NEt_2 \longrightarrow CH_2Cl{\cdot}CO{\cdot}NEt_2$$
$$\downarrow NHEt_2 \qquad (I.)$$
$$CHCl{:}C(NEt_2)_2 \quad (II.) \qquad [NMe_3{\cdot}C{:}C{\cdot}NMe_3]^{++}2Cl' \quad (IV.)$$
$$(III.)\ NHBu{\cdot}CH_2{\cdot}C(:NBu){\cdot}NHBu \qquad CH(CO_2Et)_2{\cdot}CH{:}CCl{\cdot}CH(CO_2Et)_2 \quad (V.)$$

With primary amines, *e.g.*, *iso*butylamine, the product is the substituted amidine (III), and ammonia yields chloroacetonitrile, presumably from the initial adduct ($CHCl{:}CCl{\cdot}NH_2$). Quaternary salts (*e.g.*, IV) are produced by reactions with tertiary amines, and ethyl sodiomalonate gives (V) by addition and subsequent replacement reactions.

The reactions of the simple 1-halogenoacetylenes have been investigated in some detail by various workers.[79] The carbon–halogen bond is particularly strong, and, as would be expected, the halogen atoms are more readily replaced by positive than by negative groups, the iodo-compounds being the most reactive. Sharp differences are often observed depending upon the nature of the halogen atom present and, moreover, the halogeno-alkylacetylenes and the more reactive halogeno-arylacetylenes frequently behave quite differently. With sodium in ether or benzene, sodio-acetylenes are obtained, but only the iodo-compounds form Grignard reagents by reaction with magnesium. Although they are unaffected even under most vigorous conditions by aqueous alkalis, in alcoholic solution acids and hydrocarbons are formed in proportions depending both upon the particular halogen atom

[76] V. Grignard, E. Bellet, and C. Courtot, *Ann. Chim.*, 1915, **4**, 30.
[77] *Ber.*, 1931, **64**, 1324; 1930, **63**, 1941; 1942, **75**, 1517.
[78] *Idem, ibid.*, 1943, **76**, 80, 84, 88. [79] *Inter alia*, see refs. 16, 73 and 75.

present and upon the nature of the substituent group R. The direction of the addition of water is not influenced by the presence of the halogen atom

$$R\cdot C{:}CX \xrightarrow{\text{alc. KOH}} R\cdot CH_2\cdot CO_2H + R\cdot C{:}CH$$

$$R\cdot C(OMe)_2\cdot CH_2X \xleftarrow[MeOH]{HgO;\ BF_3\text{-}Et_2O} R\cdot C{:}CX \xrightarrow[H_2SO_4]{HgSO_4} R\cdot CO\cdot CH_2X$$

(except in alkaline media, as indicated above), the aryl compounds giving halogeno-ketones with a mixture of sulphuric and acetic acids. The presence of mercuric salts, however, is necessary for the successful hydration of the alkyl analogues, and the mercuric oxide–boron fluoride catalyst in methyl alcohol yields the ketals. When the iodo-acetylenes are heated with aqueous alcoholic mercuric chloride, chloro-ketones result from a hydration and halogen replacement reaction. With potassium cyanide in aqueous methyl alcohol the cyano-ethylenic ether (VI) is readily obtained from the chloro-heptyne. No replacement occurs in inert solvents, but the observation [80]

$$C_5H_{11}\cdot C{:}CCl \xrightarrow[MeOH\text{–}Aq.]{KCN} \underset{(VI.)}{C_5H_{11}\cdot C(OMe){:}CH\cdot CN} \xleftarrow[KOH]{MeOH} \underset{(VII.)}{C_5H_{11}\cdot C{:}C\cdot CN}$$

that addition of methyl alcohol to cyanoheptyne (VII) occurs smoothly in the presence of alkali may possibly be significant.

Vinylacetylene and Related Hydrocarbons—Reactions.

The reactions of conjugated vinylacetylenes, particularly those of the parent hydrocarbon (I), are of considerable academic interest and industrial importance, and during the past fifteen years they have received detailed attention, especially in the U.S.A. and U.S.S.R. W. H. Carothers and his associates found that addition reactions with electrophilic reagents proceed by means of an initial 1 : 4-addition to the conjugated system and the resulting allene derivatives (II) isomerise more or less readily into the butadienes (III)

$$\underset{(I.)}{CH{:}C\cdot CH{:}CH_2} \xrightarrow{HX} \underset{(II.)}{CH_2{:}C{:}CH\cdot CH_2X} \longrightarrow \underset{(III.)}{CH_2{:}CX\cdot CH{:}CH_2}$$

by anionotropic rearrangements of the usual type.[81] In addition to reactions leading eventually to various 2-substituted butadienes, numerous studies involving substitution of the active hydrogen atom giving alkylvinylacetylenes, carbinols, carboxylic acids, halogen compounds and related substances have been made and both the formation of unsaturated ketones by hydration reactions and the semihydrogenation to butadienes have received much attention. Limitations of space, however, do not permit of the discussion of all of these aspects of vinylacetylene chemistry in the present Report.

The ultra-violet absorption spectra of compounds containing the conjugated vinylacetylene system (C:C·C:C) all exhibit maxima of high intensity in the range 2230—2270 A. together with characteristic inflexions at slightly

[80] C. Moureu and I. Lazennec, *Bull. Soc. chim.*, 1906, **35**, 528.

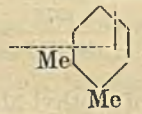
[81] E. H. Farmer, *Ann. Reports*, 1932, **29**, 108; 1933, **30**, 133; W. H. Carothers and G. J. Berchet, *J. Amer. Chem. Soc.*, 1933, **55**, 2807; J. H. Werntz, *ibid.*, 1935, **57**, 204.

longer (100 A.) wavelengths.[82] The locations of the maxima correspond closely to those of the corresponding dienes, but appreciable intensity differences are usually observed. Further examples,[83] together with the light absorption data for a number of conjugated polyene-yne systems,[84] all support the contention that replacement of one ethenoid linkage of a polyene system by an acetylenic bond has a negligible effect on the position of the absorption maxima.

The polymerisation of vinylacetylene itself appears to proceed along three quite distinct routes. In the presence of cuprous chloride a liquid linear dimer, probably (IV), is amongst the products formed, but purely thermal treatment or peroxide-catalysed polymerisation gives materials (*e.g.*, V) containing *cyclo*butane rings,[85] a dimerisation analogous to that undergone by cinnamic acid. Thirdly, when vinylacetylene is heated at 105° with a variety of reagents, especially those of an acidic nature, styrene

(IV.) $CH_2{:}CH{\cdot}CH{:}CH{\cdot}C{:}C{\cdot}CH{:}CH_2$

(V.) $CH{:}C{\cdot}CH(CH_2{-}CH_2){\cdot}C{:}CH$



(VI.)

is formed in yields of 20—50% by a reaction which can be regarded as being of the Diels–Alder type.[85] Other vinylacetylenes undergo similar dimerisations,[86] *e.g.*, 1 : 2-dimethylvinylacetylene gives the hydrocarbon (VI) at 120° in alcohol or acetic acid.

With hydrogen cyanide in the presence of cuprous chloride, vinylacetylene gives the 1-cyanobutadiene (VIII), the allene (VII) undergoing a prototropic rather than an anionotropic rearrangement, as might be expected in view of the low mobility of the cyano-group.[87] The chloro-allene corresponding to (VII) also gives (VIII) on treatment with potassium cyanide.

(I) $\xrightarrow[CuCl]{HCN}$ $[CH_2{:}C{:}CH{\cdot}CH_2{\cdot}CN]$ (VII.) $\longrightarrow$ $CH_2{:}CH{\cdot}CH{:}CH{\cdot}CN$ (VIII.)

Reactions of vinylacetylenes with alcohols apparently give different products according to whether the addition is effected by means of sodium

[82] E. R. H. Jones and J. T. McCombie, *J.*, 1943, 261.

[83] I. M. Heilbron, E. R. H. Jones, and R. A. Raphael, *J.*, 1943, 264; J. Cymerman, I. M. Heilbron, and E. R. H. Jones, *J.*, 1944, 144; I. M. Heilbron, E. R. H. Jones, R. N. Lacey, J. T. McCombie, and R. Raphael, *J.*, 1945, 77; I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *ibid.*, p. 81; J. Cymerman, I. M. Heilbron, and E. R. H. Jones, *ibid.*, p. 90.

[84] I. M. Heilbron, A. W. Johnson, E. R. H. Jones, and R. A. Raphael, *J.*, 1943, 265; I. M. Heilbron, E. R. H. Jones, and R. A. Raphael, *ibid.*, p. 268; I. M. Heilbron, E. R. H. Jones, and J. T. McCombie, *J.*, 1944, 134; I. M. Heilbron, E. R. H. Jones, and R. A. Raphael, *ibid.*, p. 136; I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *ibid.*, p. 140; see also H. Bastron, R. E. Davis, and L. W. Butz, *J. Amer. Chem. Soc.*, 1943, **65**, 973.

[85] H. B. Dykstra, *ibid.*, 1934, **56**, 1625.

[86] A. E. Favorski and A. I. Zacharova, *J. Gen. Chem. Russia*, 1937, **7**, 973; A. I. Zacharova and V. A. Bezel-Sitscheva, *ibid.*, 1941, **11**, 67.

[87] P. Kurtz and H. Schwartz, U.S.P. 2,322,696; cf. D. D. Coffmann, *J. Amer. Chem. Soc.*, 1935, **57**, 1981.

alkoxides or potassium hydroxide. With alcohols containing some sodium alkoxide at 100° the products are, rather unexpectedly, 1-alkoxy-2-butynes (IX), and it is suggested that here an allene ⟶ acetylene isomerisation (as observed with the hydrocarbons by Favorski) occurs.[88] Addition takes

$$\text{(I.)} \xrightarrow[\text{NaOMe}]{\text{MeOH}} [CH_2{:}C{:}CH{\cdot}CH_2{\cdot}OMe] \longrightarrow CH_3{\cdot}C{:}C{\cdot}CH_2{\cdot}OMe \quad \text{(IX.)}$$

place probably *via* the acetylide anion and no anionotropic rearrangement of the intermediate alkoxy-allene would be expected under the experimental conditions involved. The structure of (IX) was satisfactorily established by hydrogenation and by oxidation to acetic and methoxyacetic acids. On the other hand, it has been reported [89] that treatment of vinylacetylene with alcoholic potassium hydroxide at 150° gave 2-ethoxybutadiene (X), which was hydrolysed to methyl vinyl ketone and oxidised with permanganate

$$\text{(X.)}\quad CH_2{:}CH{\cdot}C(OEt){:}CH_2 \qquad CH_2{:}C{:}C(OMe){\cdot}CH_3 \quad \text{(XI.)}$$
$$CHMe{:}CMe{\cdot}C(OR){:}CH_2 \quad \text{(XII.)}$$

to oxalic and formic acids, and that the methyl alcohol adduct has the allene structure (XI). 1 : 2-Dimethylvinylacetylene is claimed to give analogous diene-ethers (*e.g.*, XII) under similar conditions, but although favourable ozonolysis results were obtained, no crystalline maleic anhydride adducts could be isolated.[26] Only relatively poor yields of pure materials were obtained in the processes employing potassium hydroxide, and it is of interest that the latter is reported [121] to form a potassio-derivative with vinylacetylene. Absorption spectra determinations on (X), (XI), and (XII) would have provided valuable confirmatory evidence for the assigned structures.

Addition of α-chloro-ethers in the presence of bismuth chloride, an extension of the general olefin reaction, follows the usual course. Some of the diene (XIII) is formed directly, and the allene, the major primary product,

$$\text{(I)} \xrightarrow[BiCl_3]{MeO{\cdot}CH_2Cl} CH_2Cl{\cdot}CH{:}C{:}CH{\cdot}CH_2{\cdot}OMe \xrightarrow[HCl]{CuCl} CH_2{:}CH{\cdot}CCl{:}CH{\cdot}CH_2{\cdot}OMe \quad \text{(XIII.)}$$

can readily be isomerised into it.[90] A. F. Thompson and D. M. Surgenov [91] added the triphenylmethyl radical to a vinylacetylene and obtained the crystalline di-addition product (XIV), the allene structure being established

$$CH{:}C{\cdot}CMe{:}CH_2 \xrightarrow[Hg]{CPh_3Cl} CPh_3{\cdot}CH{:}C{:}CMe{\cdot}CH_2{\cdot}CPh_3 \quad \text{(XIV.)}$$

by ozonolysis. Similar di-additions had been observed earlier with conjugated dienes. It is suggested that this reaction may be useful for the characterisation of vinylacetylenes.

[88] R. A. Jacobson, H. B. Dykstra, and W. H. Carothers, *J. Amer. Chem. Soc.*, 1934, **56**, 1169.

[89] A. L. Klebanski, L. G. Zurich, and I. M. Dolgopolski, *Bull. Acad. Sci. U.R.S.S.*, 1935, 189; I. A. Rotenberg and M. A. Favorskaja, *J. Gen. Chem. Russia*, 1936, **6**, 185.

[90] H. B. Dykstra, *J. Amer. Chem. Soc.*, 1936, **58**, 1747. [91] *Ibid.*, 1943, **65**, 486.

When a mixture of vinylacetylene and a conjugated diene is heated in the presence of a mercuric salt, hydration and Diels–Alder condensation occur, giving rise to tetrahydroacetophenones.[92] N. M. Malenok and I. M. Sologub [93] treated four vinylacetylenes of the type (XV) ($CPh{:}C{\cdot}CR{:}CHR'$) with an excess of peracetic acid. In every case only the ethenoid linkage was attacked, giving either the oxide or its fission products, depending upon the conditions employed.

The chlorination of vinylacetylene in methyl-alcoholic solution, proceeding most probably by a mechanism similar to that outlined on p. 155, gives rise

(XVI.) $CHCl{:}C(OMe){\cdot}CH{:}CH_2$ $CHCl_2{\cdot}C(OMe)_2{\cdot}CH_2{\cdot}CH_2Cl$ (XVII.)

to a complex mixture containing methyl chloride, the primary addition product (XVI) and, as final products, the trichloroketal (XVII) and the corresponding ketone.[93a]

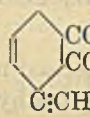
By controlled thermal polymerisation of divinylacetylene (XVIII) in the absence of air, M. E. Cupery and W. H. Carothers [94] produced an oily intermediate polymer, from which was isolated some of the dimer (XIX), giving *trans-cyclo*butane-1 : 2-dicarboxylic acid on permanganate oxidation.

$$\underset{\text{(XVIII.)}}{CH_2{:}CH{\cdot}C{:}C{\cdot}CH{:}CH_2} \longrightarrow \underset{\text{(XIX.)}}{CH_2{:}CH{\cdot}C{:}C{\cdot}\overset{CH_2}{\underset{CH_2}{CH}}{-}\overset{CH}{\underset{CH}{C}}{\cdot}C{:}C{\cdot}CH{:}CH_2}$$

Such dimerisations are reminiscent of those yielding truxinic and truxillic acids, and are in contrast to the 1 : 4-additions which have been observed with halogens and halogen acids.[95] The cyclisations undergone by various divinylacetylenes with sulphuric or formic acid were reviewed in a recent Report,[96] as also were the dual addition reactions with maleic anhydride.[97]

Ethynylbutadiene (*as.*-divinylacetylene) (XX), formed along with its isomer (XVIII) during the manufacture of vinylacetylene, polymerises with unusual rapidity and attempts to effect addition reactions frequently result in more or less extensive polymerisation. However, a vinyl-" chloroprene " can be obtained by addition of hydrogen chloride, hexa-3 : 5-dien-2-one is

(XX.) $CH_2{:}CH{\cdot}CH{:}CH{\cdot}C{:}CH$



(XXI.)

produced on hydration with acidified mercuric sulphate, and the acid (XXI) is formed by hydrolysis of the adduct obtained by heating the hydrocarbon (XX) with maleic anhydride at 75°.[20,98]

[92] U.S.P. 2,301,515.

[93] *J. Gen. Chem. Russia*, 1936, **6**, 1904; 1939, **9**, 1947; 1940, **10**, 150; 1941, **11**, 983.

[93a] A. A. Baum, R. R. Vogt, and G. F. Hennion, *J. Amer. Chem. Soc.*, 1939, **61**, 1458.

[94] *Ibid.*, 1934, **56**, 1167.

[95] W. H. Carothers *et al.*, *ibid.*, 1933, **55**, 2004, 2040, 2048.

[96] E. R. H. Jones, *Ann. Reports*, 1943, **40**, 135. [97] *Idem*, *ibid.*, p. 132.

[98] A. L. Klebanski, L. G. Zurich, and I. M. Dolgopolski, *Bull. Acad. Sci. U.R.S.S.*, 1935, **7**, 189.

II. *Acetylenic Carbinols and Glycols—Preparative Methods.*

The literature on this subject is extensive and it is only possible to give here an outline of the more important methods which have been employed and to cite representative references. Also, this account is mainly restricted to those carbinols (I) and glycols (II) which are produced when carbonyl compounds are treated with acetylenes and their derivatives containing one

$$>CO + CH{:}C^- \longrightarrow >C(OH)\cdot C{:}C^- \quad (I.) \qquad >C(OH)\cdot C{:}C\cdot C(OH)< \quad (II.)$$

or two free ethynyl groups, either in the form of their organometallic compounds or else by means of suitable condensing agents.

The most widely applicable laboratory method employs the acetylenic Grignard reagents, made by the reaction of acetylenic compounds with (usually) ethylmagnesium bromide. With substituted acetylenes, including those of the vinylacetylene [99] and ethynylbutadiene [100] types, and with almost all classes of carbonyl compounds, including the polyene aldehydes,[101] this method gives highly satisfactory results. It can also be applied, starting with the ethynylcarbinols, to the synthesis of either *s.*- or *as.*-glycols.[102] From acetylene itself, however, owing to disproportionation of the primary

$$2CH{:}C\cdot MgBr \longrightarrow C_2H_2 + BrMg\cdot C{:}C\cdot MgBr \quad (III.)$$
$$2R_1R_2CO + (III) \longrightarrow CR_1R_2(OH)\cdot C{:}C\cdot C(OH)R_1R_2 \quad (IV.)$$

product,[103] the dimagnesium bromide (III) predominates in the reaction mixture and consequently interaction with carbonyl compounds results mainly in the formation of the *s.*-glycols [104, 129] (IV), although preferential reaction with only one of the magnesium bromide groups of (III) usually produces varying amounts of the carbinols.[103] When stereoisomerism is possible, the *meso*- and the *dl*-forms of the *s.*-glycols are produced simultaneously and they can in most cases be separated by fractional crystallisation.

Acetylenylcarbinols were first prepared by J. U. Nef,[105] using suspensions of alkali-metal acetylides in ether or benzene, but this process, although reasonably well suited for reactions with substituted acetylenes (*e.g.*, CPh:CH), is unsatisfactory when applied to acetylene itself. However, by taking advantage of the solubility and reactivity of alkali-metal acetylides (R·C:CM or CH:CM) in liquid ammonia (see p. 148) a convenient and widely applicable method has recently been developed, the procedure being adopted independently and more or less simultaneously by several investigators.[106, 116]

[99] W. H. Carothers and G. J. Berchet, *J. Amer. Chem. Soc.*, 1933, **55**, 1094.

[100] J. S. Salkind and S. A. Sokis, *Chem. Zentr.*, 1938, II, 768.

[101] I. M. Heilbron, E. R. H. Jones, and R. A. Raphael, *J.*, 1944, 136.

[102] *E.g.*, C. S. Marvel *et al.*, *J. Amer. Chem. Soc.*, 1939, **61**, 2006; *J. Org. Chem.*, 1942, **7**, 93; J. Cymerman, I. M. Heilbron, A. W. Johnson, and E. R. H. Jones, *J.*, 1944, 141.

[103] H. Kleinfeller and H. Lohmann, *Ber.*, 1938, **71**, 2608.

[104] *Inter al.*, G. Dupont, *Ann. Chim.*, 1913, **30**, 485; J. S. Salkind and S. M. Labuzov, *J. Gen. Chem. Russia*, 1939, **9**, 1525.

[105] *Annalen*, 1899, **308**, 264.

[106] R. J. Levina and J. Vinogradova, *Chem. Zentr.*, 1937, I, 2369; F. C. McGrew, and R. Adams, *J. Amer. Chem. Soc.*, 1937, **59**, 1497; L. Ruzicka and K. Hofmann, *Helv. Chim. Acta*, 1937, **20**, 1280; J. Kathol, W. Logemann, and A. Serini, *Naturwiss.*, 1937, **25**, 682.

Sodium is the most suitable metal for general purposes, and the acetylides are obtained simply by addition of the acetylenic hydrocarbon to the blue solution of sodium in liquid ammonia. Because of the powerful reducing action of the latter combination, it is preferable to add the acetylene and the metal simultaneously, so that no appreciable excess of the metal is ever present in solution,[107] or, better still, to convert the sodium catalytically into sodamide (see p. 149), which reacts readily with acetylenes without effecting any reduction, the acetylides so produced being more soluble and more reactive than those prepared directly from sodium.[108] The aldehyde or ketone is added neat or in solution and after completion of the reaction the sodio-compound is decomposed by treatment with solid ammonium chloride, the ammonia is then allowed to evaporate, and the carbinol isolated by

$$R_1R_2CO + NaC{:}CR_3 \longrightarrow CR_1R_2(ONa)\cdot C{:}CR_3 \xrightarrow{NH_4Cl} CR_1R_2(OH)\cdot C{:}CR_3 + NaCl + NH_3$$

distillation or extraction. Good yields of carbinols can be obtained from saturated carbonyl compounds and the method has recently been used with polyene aldehydes [109] and also with vinylacetylenes.[110] The much less reactive αβ-unsaturated ketones give good yields of carbinols provided a large excess of highly active sodium acetylide made from sodamide is employed.[111]

Other procedures for obtaining acetylenic carbinols and glycols differ from those so far described in that the prior formation of metallic acetylides is not involved. When ethereal solutions of carbonyl compounds are treated with powdered sodamide and subsequently with acetylene, ethynylcarbinols are readily produced [112] as a consequence of the initial formation of the sodio-derivative of the enolic form of the carbonyl compound. The scope of this method, originally devised by Bayer and Co.[113] in 1915 and thereafter extensively employed, is limited, however, by the variable reactivity of the sodamide and, as are all procedures of the same type, by the adverse effect of alkaline reagents on certain carbonyl compounds, especially aldehydes. Potassium *tert.*-amyloxide and related alkoxides are also effective condensing agents,[25, 114] but difficulties may arise over the separation of simpler carbinols from the *tert.*-amyl alcohol present.

As already indicated, the Grignard reaction with acetylene itself furnishes

[107] U.S.P. 2,200,941. [108] B.P. 502,693.

[109] E. R. H. Jones and J. T. McCombie, *J.*, 1942, 733; I. M. Heilbron, E. R. H. Jones, and J. T. McCombie, *J.*, 1944, 134; I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J.*, 1945, 81.

[110] I. M. Heilbron, A. W. Johnson, E. R. H. Jones, and R. A. Raphael, *J.*, 1943, 265; I. M. Heilbron, E. R. H. Jones, and R. A. Raphael, *J.*, 1944, 136.

[111] J. Cymerman, I. M. Heilbron, and E. R. H. Jones, *J.*, 1945, 90; see also G. F. Hennion and D. J. Lieb, *J. Amer. Chem. Soc.*, 1944, **66**, 1289.

[112] *E.g.*, D. D. Coffmann, *Org. Syntheses*, 1940, **20**, 40.

[113] D.R.PP. 280,226, 286,920, 289,800; Friedländer, 1914—16, **12**, 51, 54, 55.

[114] A. F. Thompson, N. A. Milas, and I. Rovno, *J. Amer. Chem. Soc.*, 1941, **63**, 752; C. S. Marvel, D. E. Pearson, and L. A. Patterson, *ibid.*, 1940, **62**, 2659.

a satisfactory laboratory route to the *s.*-acetylenic glycols. These are also obtained as by-products in the sodium acetylide–liquid ammonia and the sodamide and alkoxide processes used for carbinol preparations. Although di-sodium acetylide cannot be prepared in liquid ammonia, the yields of glycols obtained by this method can be improved by increasing the reaction time [115] and also by avoiding an excess of acetylene, the effect of which is explained [116] by the accompanying scheme. In relation to these suggestions

$$\text{Carbinol} + C_2HNa \xleftarrow{C_2H_2} CH{:}C{\cdot}C(ONa)R_1R_2 \rightleftharpoons$$
$$\text{(V.)} \qquad CNa{:}C{\cdot}C(OH)R_1R_2 \xrightarrow{>CO} \text{Glycol}$$

it is noteworthy that good yields of glycols are stated [117] to be obtained when the sodio-compounds (V) of the carbinols are heated above 60°, and also that carbinols are produced by simple pyrolytic decomposition of the glycols[118].

Acetylene condensations employing potassium hydroxide in ether were first carried out by Favorski in 1900 and the method has been widely used with a number of variations in commercial processes for the preparation of both carbinols and glycols from ketones;[119] intermediates such as $CMe_2(OH){\cdot}OK$, are believed to be involved. It has also been used successfully [120] in condensations of ketones with vinylacetylene; in the latter case, and also with arylacetylenes, it appears likely that intermediate acetylide formation occurs.[121] A. I. Zacharova [122] has demonstrated the reversibility of the reaction and has shown that conversions such as the following,

$$CH{:}C{\cdot}C(OH)Me_2 + COPh_2 \xrightarrow[Et_2O]{KOH} \begin{cases} CPh_2(OH){\cdot}C{:}C{\cdot}C(OH)Me_2 & 40\% \\ CMe_2(OH){\cdot}C{:}C{\cdot}C(OH)Me_2 & 13\% \\ CPh_2(OH){\cdot}C{:}C{\cdot}C(OH)Ph_2 & 4\% \end{cases}$$

can be effected by potassium hydroxide. The glycols are stable to the latter under the usual experimental conditions except in the presence of ketones, exchange of radicals then taking place. A somewhat similar procedure employing finely powdered calcium carbide and potassium hydroxide in an inert solvent, devised by L. Kazarjan [123] and improved by H. A. Bruson and J. W. Kroeger,[124] is more particularly suited for the production of glycols. Since the reaction proceeds normally with benzophenone, it clearly cannot involve enolisation and evidence has been submitted which indicates the following mechanism : [125]

$$COMe_2 + KOH \longrightarrow KO{\cdot}CMe_2{\cdot}OH \xrightarrow{CaC_2} (KO{\cdot}CMe_2{\cdot}C{:})_2 + Ca(OH)_2$$

[115] J. F. Froning and G. F. Hennion, *J. Amer. Chem. Soc.*, 1940, **62**, 653.
[116] K. N. Campbell, B. K. Campbell, and L. T. Eby, *ibid.*, 1938, **60**, 2882.
[117] U.S.P. 2,162,676. [118] U.S.P. 2,175,581.
[119] U.S.PP. 2,345,170, 2,163,720, 2,161,191; B.P. 544,221; A. T. Babajan, *J. Gen. Chem. Russia*, 1940, **10**, 480.
[120] I. N. Nazarov *et al.*, *Bull. Acad. Sci. U.R.S.S.*, 1938, 683; 1940, 447; 1943, 129; A. P. Golovtschanskaja, *J. Gen. Chem. Russia*, 1940, **10**, 435.
[121] D.R.P. 712,742. [122] *J. Gen. Chem. Russia*, 1941, **11**, 939.
[123] *Ibid.*, 1934, **4**, 1347; 1937, **7**, 956.
[124] *J. Amer. Chem. Soc.*, 1940, **62**, 36; U.S.P. 2,250,445.
[125] A. T. Babajan, *J. Gen. Chem. Russia*, 1938, **8**, 602.

Recently, a truly catalytic procedure for the condensation of aldehydes and ketones with acetylene has been developed [126] according to which a liquid carbonyl compound, or a solution in an inert solvent, is treated with a mixture of acetylene and nitrogen under pressure at 100—130° in the presence of a catalyst consisting of cuprous or a similar acetylide supported on kieselguhr. Depending upon the proportions of reactants utilised, either carbinols or glycols [especially butynediol, $CH_2(OH)\cdot C{:}C\cdot CH_2\cdot OH$] are obtained, and the process can also be adapted to the production of *as.*-glycols by condensing carbonyl compounds with ethynylcarbinols.[127]

The preparation of diacetylenic glycols [$> C(OH)\cdot C{:}C\cdot C{:}C\cdot C(OH) <$] from diacetylene by the Grignard method, first studied by F. Strauss, L. Kollek, and H. Hauptmann,[128] has been extensively applied by R. Kuhn and his co-workers in their studies on conjugated polyenes and cumulenes.[129] The formation of the glycols by oxidation of metallic derivatives of the ethynylcarbinols has been mentioned earlier in this Report (p. 151).

The action of acetylenic (or vinylacetylenic) Grignard reagents on esters produces diacetylenic carbinols (VI),[130] which are also obtained together

$$CH_3\cdot CO_2Et + 2R\cdot C{:}C\cdot MgBr \longrightarrow (R\cdot C{:}C)_2C(OH)Me \quad \text{(VI.)}$$

$$R\cdot C{:}C\cdot MgBr + \underset{\diagdown\,\diagup}{CH_2\cdot CH_2}\,O \longrightarrow R\cdot C{:}C\cdot CH_2\cdot CH_2\cdot OH \quad \text{(VII.)}$$

with acetylenic ketones ($Me\cdot CO\cdot C{:}CR$) by employing acetic anhydride.[131] Both the Grignard reagents and the sodio-derivatives of acetylenic hydrocarbons react with ethylene oxide, yielding carbinols of type (VII).[132]

Acetylenic Carbinols and Glycols—Reactions.

Interest in the reactions of the carbinols, especially the readily available simpler members, has arisen largely because of the possibility of converting them into polymerisable materials. Earlier studies, mainly with the benzenoid carbinols, were concerned with free radicals and radical stability and the formation and structure of rubrenes.[133] Within recent years the ethynylcarbinols derived from steroid ketones have attracted much attention, since the ethynylcarbinol (pregneninolone) of androstenedione was found to exhibit considerable progesterone-like activity. Also, investigation of the reactions of these carbinols has led to the discovery of a novel ring enlarge-

[126] B.P. 508,062; U.S.P. 2,300,969.

[127] U.S.P. 2,238,471. [128] *Ber.*, 1930, **63**, 1886.

[129] R. Kuhn and K. Wallenfels, *Ber.*, 1938, **71**, 783, 1889; R. Kuhn and G. Platzer, *Ber.*, 1940, **73**, 1410.

[130] J. S. Salkind and V. Pletz, *J. Gen. Chem. Russia*, 1934, **4**, 1088; G. I. Iositch *et al.*, *J. Russ. Phys. Chem. Soc.*, 1910, **42**, 1082, 1492; P. L. Viguier, *Ann. Chim.*, 1913, **28**, 525; C. K. Liang, *Bull. Soc. chim.*, 1933, **53**, 33; H. B. Gillespie and C. S. Marvel, *J. Amer. Chem. Soc.*, 1930, **52**, 3368; S. S. Rossander and C. S. Marvel, *ibid.*, 1929, **51**, 932.

[131] J. W. Kroeger and J. A. Nieuwland, *ibid.*, 1936, **58**, 1861.

[132] J. P. Danehy, R. R. Vogt, and J. A. Nieuwland, *ibid.*, 1934, **56**, 2790; 1935, **57**, 2327; G. B. Bachman, *ibid.*, 1935, **57**, 382; U.S.P. 2,106,182.

[133] See R. D. Haworth, *Ann. Reports*, 1937, **34**, 389; G. A. R. Kon, *ibid.*, 1932, **29**, 173.

ment.[134] The semihydrogenation of the acetylenic linkage in both carbinols and glycols has been reviewed [52] and their dehydration to conjugated vinyl- and divinyl-acetylenes, respectively, has been referred to earlier in this Report (p. 152). A review of certain aspects of the chemistry of the acetylenic alcohols has been written by A. W. Johnson.[135]

In 1922 K. H. Meyer and K. Schuster[136] discovered a rearrangement which has since been found to underlie a great many of the reactions of acetylenylcarbinols and related compounds. They found that the carbinol (I) was smoothly converted into the unsaturated ketone (III) on treatment with a

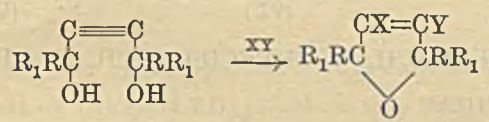
$$\underset{\text{(I.)}}{CPh_2(OH)\cdot C{:}CPh} \longrightarrow \underset{\text{(II.)}}{[CPh_2{:}C{:}C(OH)Ph]} \longrightarrow \underset{\text{(III.)}}{CPh_2{:}CH\cdot COPh}$$

variety of reagents, including sulphuric acid, thionyl chloride, and acetic anhydride; they proved conclusively that no phenyl group migration occurred and the allene derivative (II) was postulated as an intermediate. Many more and varied examples of this rearrangement have emerged during

$$>CX\cdot C{:}C^- \rightleftharpoons >C{:}C{:}CX^- \longrightarrow >C{:}CH\cdot CO^-$$

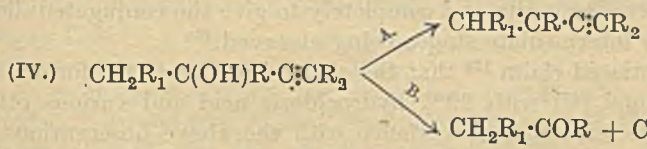
the last twenty years and it is now generally appreciated that the above anionotropic system is readily established in many reactions with acetylenic compounds, usually when acidic reagents are employed.

In all reactions with the acetylenic glycols, especially those of the ditertiary type, where addition to the triple bond can conceivably take place, *i.e.*, hydration, halogenation, hydrogenation, etc., in addition to the possible



occurrence of Meyer–Schuster rearrangements when reagents of an acidic character are used, a further complication is introduced by the facile dehydration of the primary addition products to dihydrofuran derivatives.

Fission with Alkalis.—The fission of acetylenic carbinols and glycols into acetylenes and carbonyl compounds by the action of alkaline reagents, first observed by Henry in 1875 with propargyl alcohol, has recently been studied in somewhat greater detail. With 50—60% potassium hydroxide solution, carbinols of type (IV) generally give ketones and acetylenes (route B), independently of the nature of the substituent groups. In the vapour



phase over alumina at 230°, however, a more selective action is observed, the nature of the group R_2 (in IV) having a major influence on the mode of

[134] See F. S. Spring, *Ann. Reports*, 1940, **37**, 332; 1943, **40**, 156.
[135] *Sci. J. Roy. Coll. Sci.*, 1942, **12**, 46. [136] *Ber.*, 1922, **55**, 819.

fission of the carbinol. When R and R_1 are alkyl groups, smooth dehydration occurs (route A) except when R_2 is H, Me or Ph, in which cases the predominating reaction is fission according to route B.[25, 27] The *tert.*-acetylenic glycols (IV*a*) also undergo fission on heating with alkalis to give ketones together with either carbinols or acetylene. With potassium hydroxide, no

$$\underset{\text{(IV}a\text{).}}{CRR_1(OH)\cdot C{:}C\cdot C(OH)RR_1} \begin{cases} \rightarrow CRR_1(OH)\cdot C{:}CH + COR_1 \\ \rightarrow CH{:}CH + 2COR_1 \end{cases}$$

stepwise degradation, such as occurs when magnesium oxide or potassium carbonate is used, is observed. With the latter reagent at 150—170°, A. T. Babajan [137] found that, when R and R_1 were alkyl groups, the carbinol was formed almost exclusively and that, when either or both R and R_1 were aryl groups, decomposition occurred mainly in the alternative manner.

Reactions with Halogen Acids.—Rapid treatment of ethynylcarbinols with concentrated hydrochloric acid or with dry hydrogen chloride results in replacement halogenation of the hydroxyl group.[10, 116] With dimethylethynylcarbinol (V), better yields of the chloro-compound (VI) are obtained with hydrochloric acid in the presence of cuprous and ammonium chlorides. If the treatment under these conditions is prolonged, the chloro-compound is gradually isomerised, first to the allene (VII) and then, much more slowly,

$$\underset{\text{(V.)}}{CH{:}C\cdot C(OH)Me_2} \xrightleftharpoons[CaCO_3]{HCl} \underset{\text{(VI.)}}{CH{:}C\cdot CClMe_2} \xrightleftharpoons[CaCO_3]{HCl\text{–}CuCl} \underset{\text{(VII.)}}{CHCl{:}C{:}CMe_2}$$

$$\underset{\text{(VIII.)}}{CHCl{:}CH\cdot CMe{:}CH_2} \qquad \underset{\text{(IX.)}}{CHBr{:}CH\cdot CMe{:}CH_2} \qquad \underset{\text{(X.)}}{CH_2{:}CMe\cdot CCl{:}CH_2}$$

(HCl–CuCl ⇌ $CaCO_3$ between (VII) and (VIII).)

to the chloro-diene (VIII). The latter forms an adduct, with elimination of the halogen atom, with maleic anhydride. The formation of the allene (VII), clearly an example of the Meyer–Schuster rearrangement, is partially reversed on heating with an aqueous suspension of calcium carbonate, although much conversion into the diene (VIII) ensues by a prototropic rearrangement. Some methylcrotonaldehyde is also formed, possibly by hydrolysis of (VII). Similar, but much slower reactions take place with the higher homologues of dimethylethynylcarbinol. No matter how the conditions are varied, the reaction of (V) with hydrobromic acid, catalysed by cuprous salts, proceeds rapidly and completely to give the conjugated diene (IX), without any intermediate stages being observed.[138]

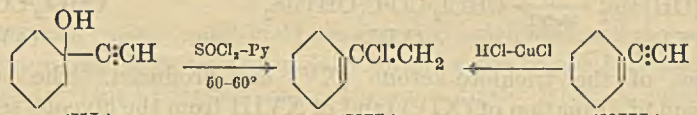
The unsubstantiated claim [139] that the chloro-isoprene (X) is formed by treating the carbinol (V) with 29% hydrochloric acid and various other chlorinating agents, although at variance with the above observations of

[137] A. T. Babajan, *J. Gen. Chem. Russia*, 1938, **8**, 578; 1939, **9**, 396.

[138] T. A. Favorskaja *et al.*, *ibid.*, 1939, **9**, 386, 1237; 1940, **10**, 446, 451, 461; 1942, **12**, 638. For similar reactions, see T. D. Nagibina, *ibid.*, 1940, **10**, 427; A. I. Zacharova, *ibid.*, 1938, **8**, 1224.

[139] U.S.P. 2,274,611.

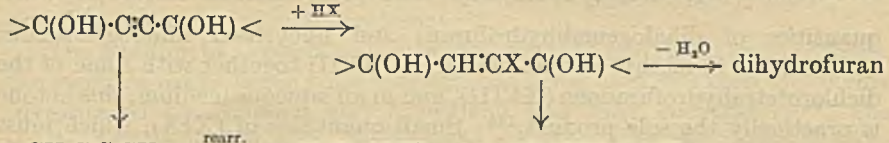
Favorskaya, is supported by the work of C. D. Hurd and R. N. Jones [140] on similar reactions with 1-ethynyl*cyclo*hexanol (XI). With thionyl chloride and pyridine, a chloro-hydrocarbon is obtained which, according to ozonolysis and other experiments, can only have the structure (XII), and which appears to be identical with the product formed [141] by the cuprous chloride



(XI.) (XII.) (XIII.)

catalysed addition of hydrogen chloride to 1-ethynyl*cyclo*hexene (XIII). Hurd and Jones suggest that the latter is an intermediate in the formation of (XII). The presence of some of this vinylacetylene in the reaction product, and the closely analogous conversion of the carbinol (XI) into acetyl*cyclo*hexene on heating with formic acid (see p. 173), lend weight to this explanation. Cuprous salts must exert a powerful directing influence in reactions of ethynylcarbinols with halogen acids.

Interaction of acetylenic glycols with hydrobromic or hydriodic acid results initially in replacement halogenation of the hydroxyl groups and/or addition to the triple bond. In the latter event, the hydroxyl groups may



then be replaced by halogens, or cyclisation to a dihydrofuran may occur. When initial substitution takes place, the dihalogeno-acetylene may undergo a simple or a dual Meyer–Schuster rearrangement at a rate depending upon the particular halogen acid employed, or the acid may add to the acetylenic linkage. In consequence of this multiplicity of reactions, hardly any entirely satisfactory studies are to be found in the literature, in all cases a complex product is obtained, and the isolation and characterisation of pure individuals is difficult.[142] It is unfortunate that the action of hydrochloric acid has received but little attention,[143] since some of the above complications would undoubtedly be minimised, as was found to be the case in the halogenation of the acetylenic glycols derived from αβ-unsaturated aldehydes [144] (see p. 176).

[140] *J. Amer. Chem. Soc.*, 1934, **56**, 1924.

[141] W. H. Carothers and D. D. Coffman, *ibid.*, 1932, **54**, 4075.

[142] J. S. Salkind *et al.*, *J. Russ. Phys. Chem. Soc.*, 1926, **58**, 1039, 1044, 1052; 1927, **59**, 283; 1929, **61**, 803; 1930, **62**, 1011; *Ber.*, 1926, **59**, 1936; 1928, **61**, 2306; V. N. Krestinski, *Ber.*, 1926, **59**, 1030; *J. Russ. Phys. Chem. Soc.*, 1929, **61**, 1691.

[143] Cf. G. Dupont, *Compt. rend.*, 1911, **152**, 198; J. R. Johnson, *J. Amer. Chem. Soc.*, 1941, **63**, 2282.

[144] I. M. Heilbron, E. R. H. Jones, R. N. Lacey, J. T. McCombie and R. Raphael, *J.*, 1945, 77.

Halogenation.—Chlorination of dimethylethynylcarbinol (V) in inert solvents results simply in the formation of the di- and the tetra-chloro-addition product.[145] In methyl alcohol at 60°, the dichloroketo-alcohol (XIV) is also formed and on chlorination in aqueous solution considerable

$$\underset{\text{(V.)}}{CH{:}C{\cdot}C(OH)Me_2} \xrightarrow[MeOH]{Cl_2} \underset{\text{(XIV.)}}{CHCl_2{\cdot}CO{\cdot}C(OH)Me_2} \qquad \underset{\text{(XV.)}}{CHCl_2{\cdot}CO{\cdot}CClMe_2}$$

quantities of the trichloro-ketone (XV) are produced. The suggested mechanism of formation of (XIV) (and of XVIII from the glycol; see below) involves the elimination of methyl chloride, and is similar to that proposed earlier for the chlorination of 1-hexyne in methyl alcohol (p. 155).

Chlorination [145] or bromination [146] of the acetylene glycol (XVI) from acetone in inert solvents is accompanied by much cyclodehydration, giving the tetrahydrofuran derivatives (XVII) in high yields, together with small

$$\underset{\text{XVI.)}}{CMe_2(OH){\cdot}C{:}C{\cdot}C(OH)Me_2} \xrightarrow[-H_2O]{X_2} \underset{\text{(XVII.)}}{\begin{array}{c}CX_2{-}CX_2\\ CMe_2\ CMe_2\\ \diagdown\ \diagup\\ O\end{array}} \qquad \underset{\text{(XVIII.)}}{\begin{array}{c}CO{-}CCl_2\\ CMe_2\ CMe_2\\ \diagdown\ \diagup\\ O\end{array}}$$

(XIX.) $CMe_2(OH){\cdot}CO{\cdot}CCl_2{\cdot}C(OH)Me_2$ $\quad$ $R{\cdot}CH(OH){\cdot}CBr{:}CBr{\cdot}CH(OH)R$ (XX.)

quantities of dihalogenodihydrofurans and uncyclised dihalogeno-diols. Chlorination in methyl alcohol also gives (XVII) together with some of the dichlorotetrahydrofuranone (XVIII), and in an aqueous medium, this ketone is practically the sole product.[145] Small quantities of (XIX), which must be the precursor of the cyclic ketone (XVIII), are isolated from chlorinations effected in methyl-alcoholic and aqueous solutions [145] and also when the glycol (XVI) is treated with *N*-chlorourea.[147] With the di-secondary glycols, G. Dupont [148] observed that the addition of two bromine atoms proceeds quite smoothly without cyclisation, giving well-characterised dibromo-ethylenic glycols (XX). Hydrofuran formation occurs most readily on iodination.[149]

Hydration.—The direct conversion of acetylenylcarbinols into hydroxy-ketones is best carried out by using mercuric sulphate and dilute sulphuric acid.[150] As in all cases of acetylenic bond hydration, excepting of course

$$CH{:}C{\cdot}C(OH)R_2 \xrightarrow[H_2SO_4]{HgSO_4} CH_3{\cdot}CO{\cdot}C(OH)R_2$$

acetylene itself, no authenticated examples of aldehyde formation are known. Under more anhydrous conditions, heating with 90% formic acid or hydrated

[145] G. F. Hennion and G. M. Wolf, *J. Amer. Chem. Soc.*, 1940, **62**, 1368; see also A. A. Petrov, *J. Gen. Chem. Russia*, 1943, **13**, 331.

[146] A. A. Kruglov, *ibid.*, 1936, **6**, 925.

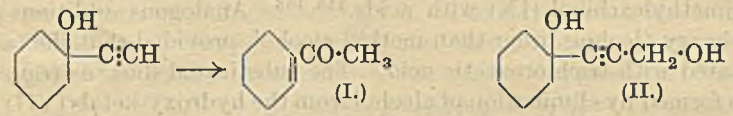
[147] V. N. Krestinski and N. I. Summ, *ibid.*, 1940, **10**, 927.

[148] *Ann. Chim.*, 1913, **30**, 519. See also ref. 146.

[149] A. A. Kruglov, *J. Gen. Chem. Russia*, 1937, **7**, 2605.

[150] *Inter al.*, H. Scheibler and A. Fischer, *Ber.*, 1922, **55**, 2903.

oxalic acid, αβ-unsaturated ketones (I) are obtained.[151] The reaction cannot be of the Meyer–Schuster type as was originally believed to be the case, as



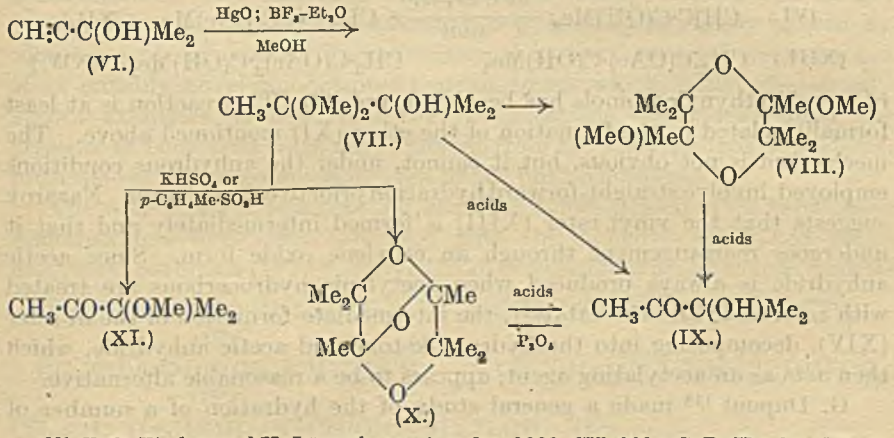
such a rearrangement would yield unsaturated aldehydes, and it would seem best to formulate it as involving dehydration prior to the hydration of the triple bond. This hydration may occur *via* a diester which is subsequently hydrolysed or decomposed to the ketone (cf. p. 174). The facile dehydration of the tertiary hydroxyl group of the analogous glycol (II) on treatment either with hot formic acid or with benzoyl chloride in pyridine [152] renders the above mechanism even more feasible.

The action of formic acid on the acetylenic glycol (III) gives a product from which the α-diketone (IV) and the hydroxy-ethylenic ketone (V) are isolated, these compounds undoubtedly arising from dual and single Meyer–

$$CMe_2{:}CH{\cdot}CO{\cdot}C(OH)Me_2 \;(V.) \longleftarrow CMe_2(OH){\cdot}C{:}C{\cdot}C(OH)Me_2 \;(III.) \longrightarrow CHMe_2{\cdot}CO{\cdot}CO{\cdot}CHMe_2 \;(IV.)$$

Schuster isomerisations respectively.[153] The corresponding glycol from diethyl ketone behaves quite differently, suffering dehydration to the divinylacetylene on similar treatment.[154]

The addition of alcohols and carboxylic acids to acetylenic hydrocarbons, the mercuric oxide–boron trifluoride catalyst being used, has been thoroughly investigated by Hennion and Nieuwland and their co-workers.[155] The similar but somewhat more complicated reactions with the carbinols have now been studied. Treatment of dimethylethynylcarbinol (VI) with methyl



[151] F. G. Fischer and K. Löwenburg, *Annalen*, 1929, **475**, 183; C. D. Hurd and R. E. Christ, *J. Amer. Chem. Soc.*, 1937, **59**, 118.

[152] K. Zeile and H. Meyer, *Ber.*, 1942, **75**, 356.

[153] V. N. Krestinski and L. I. Baschenova-Koslovskaja, *Ber.*, 1933, **66**, 97.

[154] V. N. Krestinski and N. I. Summ, *J. Gen. Chem. Russia*, 1937, **7**, 440.

[155] For summary, see Ref. 51.

alcohol and the catalyst gives mainly the hydroxy-ketal (VII), accompanied by some of the dioxan (VIII), both products being readily hydrolysed to acetyldimethylcarbinol (IX) with acids.[115, 156] Analogous additions ensue with primary alcohols other than methyl alcohol, provided that the catalyst is activated with trichloroacetic acid. The substituted dioxans (*e.g.*, VIII) are also formed by elimination of alcohol from the hydroxy-ketals (VII) under the influence either of the catalyst [156] or of acids.[115] Elimination of methyl alcohol from (VII) under more vigorous conditions, by heating with toluene-*p*-sulphonic acid,[115] formic acid or potassium hydrogen sulphate,[157] yields mainly (X), previously obtained, but incorrectly formulated, by Scheibler and Fischer [150] by treatment of the hydroxy-ketone (IX) with phosphorus pentoxide. When potassium hydrogen sulphate is used, some of the keto-ether (XI) is isolated, its formation being attributed to the spontaneous isomerisation of the intermediate vinyl ether, CH_2:C(OMe)·C(OH)Me_2.

The hydroxy-ketals, corresponding to (VII), obtained from methyl-ethyl- and diethyl-ethynylcarbinols are cyclised to dioxan derivatives (such as VIII) only with great difficulty and the failure to obtain substances of the type of (X) and (XI) on treatment with potassium hydrogen sulphate is taken as indicating that such compounds arise *via* the dioxans. In these cases stable vinyl ethers such as CH_2:C(OMe)·C(OH)MeEt are said to be formed.[158] The catalysed addition of methyl alcohol to both primary and secondary ethynylcarbinols results exclusively in the production of dioxan derivatives related to (VIII).[159]

When dimethylethynylcarbinol (VI) reacts with carboxylic acids and the boron trifluoride–mercuric oxide catalyst, the products, rather unexpectedly, are esters (XII) of acetyldimethylcarbinol.[115, 159, 160] (A similar behaviour

$$\text{(VI.)}\quad CH{:}C{\cdot}C(OH)Me_2 \xrightarrow[AcOH]{HgO:\ BF_3\text{-}Et_2O} CH_3{\cdot}CO{\cdot}C(OAc)Me_2 \quad \text{(XII.)}$$

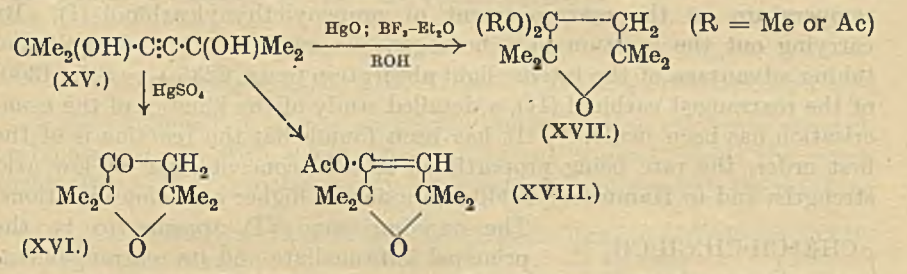
(XIII.) CH_2:C(OAc)·C(OH)Me_2 $\qquad$ CH_3·C(OAc)$_2$·C(OH)Me_2 (XIV.)

of steroid ethynylcarbinols has been observed.[161]) The reaction is at least formally related to the formation of the ether (XI) mentioned above. The mechanism is not obvious, but it cannot, under the anhydrous conditions employed, involve straight-forward hydration prior to esterification. Nazarov suggests that the vinyl ester (XIII) is formed intermediately and that it undergoes rearrangement through an ethylene oxide form. Since acetic anhydride is always produced when acetylenic hydrocarbons are treated with acetic acid and the catalyst, the intermediate formation of the di-ester (XIV), decomposing into the hydroxy-ketone and acetic anhydride, which then acts as an acetylating agent, appears to be a reasonable alternative.

G. Dupont [148] made a general study of the hydration of a number of

[156] I. N. Nazarov and A. N. Elizarova, *Bull. Acad. Sci. U.R.S.S.*, 1940, 195.
[157] *Idem, ibid.*, p. 203.
[158] I. N. Nazarov and T. D. Nagibina, *ibid.*, 1941, 303.
[159] G. F. Hennion and W. S. Murray, *J. Amer. Chem. Soc.*, 1942, **64**, 1220.
[160] I. N. Nazarov and A. N. Elizarova, *Bull. Acad. Sci. U.R.S.S.*, 1940, 215.
[161] *Inter al.*, L. Ruzicka, K. Gatzi, and T. Reichstein, *Helv. Chim. Acta*, 1939, **22**, 626.

acetylenic glycols on heating with mercuric sulphate, and he observed almost theoretical conversions into tetrahydrofuranone derivatives; the glycol (XV) from acetone gave the ketone (XVI). The formation of the same compound (XVI) (80% yields) by using the boron fluoride–mercuric oxide catalyst in either methyl alcohol or acetic acid [115] appears, at first sight,



rather abnormal. It is clear, however, that the water eliminated during the cyclisation must be responsible for the hydrolysis of the intermediate ketal or diester (XVII). Under more vigorous conditions, with boron trifluoride and mercuric acetate in acetic acid at 115°, the ketone (XVI) and the acetoxydihydrofuran (XVIII) were both isolated.[162]

Anionotropic Rearrangements.—During an investigation of the reactions of the ethylenic ethynylcarbinols prepared from αβ-unsaturated aldehydes, E. R. H. Jones and J. T. McCombie [82] observed the irreversible isomerisation of propenylethynylcarbinol (I) to the conjugated acetylenylvinylcarbinol (II) on shaking with dilute acids. A number of similar examples of this

(I.) $CH{:}C\cdot CH(OH)\cdot CH{:}CH\cdot CH_3 \xrightarrow{\text{acids}} CH{:}C\cdot CH{:}CH\cdot CH(OH)\cdot CH_3$ (II.)

transformation were described, the structures of the rearranged carbinols, suggested by their absorption spectra (see p. 161), being rigidly proved by complete hydrogenation. These investigations have revealed the existence of an entirely novel anionotropic rearrangement which, unlike the already known examples, is free from the complications consequent upon the presence of benzenoid rings and, different from the known aliphatic systems, proceeds readily to completion even under mild conditions.

The wide scope of this reaction has been demonstrated in that analogous isomerisations have been observed with the hexynyl- (III) and vinylacetylenyl-carbinols (IV) [163] derived from crotonaldehyde, as well as with its

$C_4H_9\cdot C{:}C\cdot CH(OH)\cdot CH{:}CH\cdot CH_3 \longrightarrow C_4H_9\cdot C{:}C\cdot CH{:}CH\cdot CH(OH)\cdot CH_3$
(III.)

$CH_2{:}CH\cdot C{:}C\cdot CH(OH)\cdot CH{:}CH\cdot CH_3 \longrightarrow CH_2{:}CH\cdot C{:}C\cdot CH{:}CH\cdot CH(OH)\cdot CH_3$
(IV.)

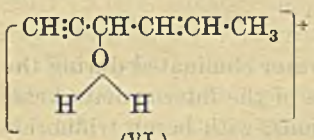
$CH_3\cdot CH{:}CH\cdot CH(OH)\cdot C{:}C\cdot CH(OH)\cdot CH{:}CH\cdot CH_3$ (V.) $\longrightarrow$
$CH_3\cdot CH(OH)\cdot CH{:}CH\cdot C{:}C\cdot CH{:}CH\cdot CH(OH)\cdot CH_3$

[162] J. S. Salkind and V. I. Baranov, *J. Gen. Chem. Russia*, 1940, **10**, 1432.

[163] I. M. Heilbron, E. R. H. Jones, and R. A. Raphael, *J.*, 1943, 264; I. M. Heilbron, A. W. Johnson, E. R. H. Jones, and R. A. Raphael, *ibid.*, p. 265; I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J.*, 1944, 140.

acetylenic glycol (V),[164] the conjugated divinylacetylenyl system being produced in the latter cases. Further, the hexynylcarbinols,[156] and more recently the ethynylcarbinols,[111] from αβ-unsaturated ketones have been shown to rearrange with considerable facility.

Early observations [82] indicated the pronounced effect of acid strength and temperature on the rearrangement of propenylethynylcarbinol (I). By carrying out the reaction in a homogeneous medium (20% alcohol) and taking advantage of the intense light absorption (max. 2235 A.; $E^{1\%}_{1\,cm.}$ 1300) of the rearranged carbinol (II), a detailed study of the kinetics of the isomerisation has been made.[166] It has been found that the reaction is of the first order, the rate being proportional to acid concentration at low acid strengths and to Hammett's acidity function at higher acid concentrations.



(VI.)

The oxonium ion (VI) appears to be the principal intermediate and its rearrangement, involving the intramolecular migration of a neutral water molecule, is suggested as the rate-determining step. During the progress of the above preparative studies [82, 111, 163, 165] considerable variations in the case of isomerisation were encountered and, although tentative suggestions were made as to the possible effects produced by various substituent groups, it was realised that owing to solubility differences anomalies were inevitable. A wide range of kinetic studies in homogeneous media [167] reveals the profound effect of increasing alkyl substitution

(VII.) $CR_1R_2{:}CH{\cdot}CR_3(OH){\cdot}C{:}CR_4$ $CH{:}C{\cdot}CH{:}CH{\cdot}CHCl{\cdot}CH_3$ (VIII.)

on rate, the latter varying from 1 for vinylethynylcarbinol (VII; $R_1 = R_2 = R_3 = R_4 = H$) to $1{\cdot}4 \times 10^9$ for the hexynylcarbinol (VII; $R_1 = R_2 = R_3 = Me$, $R_4 = Bu$) from mesityl oxide.

As might be expected from the anionotropic nature of the isomerisation, the action of cold concentrated hydrochloric acid rapidly yields the highly reactive isomerised chloro-compounds (*e.g.*, VIII), which are also produced, in poorer yields, by similar treatment of the rearranged carbinols (*e.g.*, II).[144] Ethers of (II) are obtained on rearrangement of the parent alcohol (I) in alcoholic sulphuric acid.[168]

The extension [169] of the isomerisation to the acetylenic carbinols and glycols from the polyene aldehydes, sorbaldehyde and octatrienal, represents a discovery of considerable interest in the polyene field, although the difficult problem of the semihydrogenation of the triple bond in these highly unsaturated carbinols and glycols has yet to be solved before compounds of the carotenoid type can be synthesised. The glycol (IX) from octatrienal, a

[164] I. M. Heilbron, E. R. H. Jones, and R. A. Raphael, *J.*, 1943, 268.
[165] J. Cymerman, I. M. Heilbron, and E. R. H. Jones, *J.*, 1944, 144.
[166] E. A. Braude and E. R. H. Jones, *ibid.*, p. 436; E. A. Braude, *ibid.*, p. 443.
[167] *Idem*, *ibid.*, in the press.
[168] I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J.*, 1945, 81.
[169] I. M. Heilbron, E. R. H. Jones, and J. T. McCombie, *J.*, 1944, 134; I. M. Heilbron, E. R. H. Jones, and R. A. Raphael *ibid.*, p. 136.

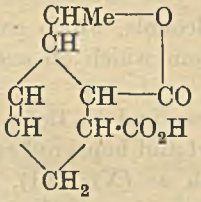
colourless crystalline solid, is transformed under exceedingly mild conditions (in cold acetone with dilute acid) into the fully conjugated orange-coloured hexaenyne glycol (X), the saturated diol obtained on hydrogenation being oxidised to thapsic acid.

$$\underset{\text{(IX.)}}{CH_3\cdot[CH{:}CH]_3\cdot CH(OH)\cdot C{:}C\cdot CH(OH)\cdot[CH{:}CH]_3\cdot CH_3} \longrightarrow CH_3\cdot CH(OH)\cdot[CH{:}CH]_3\cdot C{:}C\cdot[CH{:}CH]_3\cdot CH(OH)\cdot CH_3 \quad \text{(X.)}$$

Semihydrogenation of the acetylenic linkage in the ethylenic ethynylcarbinols (*e.g.*, I), with a supported palladium catalyst, yields the vinylcarbinols (*e.g.*, XI), which are even more readily isomerised,[170] the resulting

$$\underset{\text{(XI.)}}{CH_2{:}CH\cdot CH(OH)\cdot CH{:}CH\cdot CH_3} \xrightarrow{\text{acids}} CH_2{:}CH\cdot CH{:}CH\cdot CH(OH)\cdot CH_3 \quad \text{(XII.)}$$

$$\xrightarrow{\text{maleic anhydride}}$$



(XIII.)

dienols (XII) giving rise with maleic anhydride to exceedingly stable lactonic acids (XIII).

Reactions of Acetylenic Chlorohydrins.—Acetylenic alcohols, prepared from halogenated aldehydes and ketones by the Grignard method, undergo some reactions of general interest. The carbinol (I) from chloroacetone, which can be hydrated in the presence of mercuric salts to the ketone (II), is converted by Lespieau's method into the oxide (III). Two stereoisomeric dioxides are similarly obtained from the acetylenic glycol. Heating with

$$\underset{\text{(II.)}}{CH_2Cl\cdot C(OH)Me\cdot CO\cdot CH_3} \underset{H_2SO_4}{\overset{HgSO_4}{\longleftarrow}} \underset{\text{(I.)}}{CH_2Cl\cdot C(OH)Me\cdot C{:}CH} \xrightarrow{KOH\text{–}Et_2O}$$

$$\underset{\text{(III.)}}{CH_2\cdot CMe\cdot C{:}CH} \xrightarrow[ZnCl_2]{\text{Heat}} \underset{\text{(IV.)}}{CHO\cdot CHMe\cdot C{:}CH}$$

$$\underset{\text{(V.)}}{CMe_3\cdot C{:}C\cdot CMe\cdot CH_2} \xrightarrow[ZnCl_2]{\text{Heat}} CMe_3\cdot CH{:}C{:}CMe\cdot CHO \quad \text{(VI.)}$$

zinc chloride isomerises (III) in poor yield into the acetylenic aldehyde (IV),[171] but on similar treatment of the substituted acetylenic oxide (V), an aldehyde is obtained the allene structure (VI) of which is proved by oxidation experiments.[172]

[170] I. M. Heilbron, E. R. H. Jones, J. T. McCombie, and B. C. L. Weedon, *J.*, 1945, 84.
[171] N. A. Gerschtein, *J. Gen. Chem. Russia*, 1942, **12**, 132.
[172] A. E. Favorski and P. A. Tichomolov, *ibid.*, 1940, **10**, 1501.

As a culmination of a long series of fundamental researches on acetylenic carbinols and glycols, R. Lespieau [173] achieved the synthesis of the pentahydric sugar alcohols, adonitol and *dl*-arabitol, and the hexahydric *dl*-dulcitol and *allo*dulcitol (allitol). The ethynylcarbinol of dichloropropaldehyde was converted *via* the oxide into the triol (VII). After semihydrogenation of the acetylenic bond, hydroxylation with silver chlorate–osmium tetroxide gave a mixture of two pentols (VIII), the two isomers mentioned above being isolated *via* their penta-acetates. By a similar series of reactions the glycol (IX) from chloroacetaldehyde was converted into a mixture of

$$CH_2Cl{\cdot}CHCl{\cdot}CH(OH){\cdot}C{:}CH \xrightarrow[KOH]{10\%\ aq.} CH_2Cl{\cdot}\overset{O}{\overset{\diup}{C}}H{\cdot}\overset{O}{\overset{\diup}{C}}H{\cdot}C{:}CH \xrightarrow{H_2O}$$

$$\text{(VII.)}\ CH_2(OH){\cdot}[CH{\cdot}OH]_2{\cdot}C{:}CH \xrightarrow[AgClO_3\text{–}OsO_4]{H_2\text{–}Pd;} CH_2(OH){\cdot}[CH{\cdot}OH]_3{\cdot}CH_2{\cdot}OH\ \text{(VIII.)}$$

$$\text{(IX.)}\ CH_2Cl{\cdot}CH(OH){\cdot}C{:}C{\cdot}CH(OH){\cdot}CH_2Cl \longrightarrow CH_2(OH){\cdot}[CH{\cdot}OH]_4{\cdot}CH_2{\cdot}OH$$

hexahydric alcohols, which gave *allo*dulcitol (allitol) directly by crystallisation, and from which on acetylation the hexa-acetate of *dl*-dulcitol was obtained.

M. Tiffeneau and Y. Deux [174] have studied the migratory ability of the phenylethynyl and heptynyl radicals in the hydrobenzoin rearrangement of carbinols such as (X), (XI), and (XII). The organo-metallic complexes obtained by reaction of the appropriate chloro-ketone with the Grignard reagent from the hydrocarbon are isomerised to acetylenic ketones at 110°; the yields, however, do not appear to be good. The structures of the ketones

$$\text{(X.)}\ Ph{\cdot}C{:}\overset{*}{C}{\cdot}CR(OH){\cdot}CH_2Cl \longrightarrow R{\cdot}CO{\cdot}CH_2{\cdot}C{:}CPh\ (R = Me, Et, Ph)$$

$$\text{(XI.)}\ C_5H_{11}{\cdot}C{:}C{\cdot}C\overset{*}{R}(OH){\cdot}CH_2Cl \longrightarrow C_5H_{11}{\cdot}C{:}C{\cdot}CO{\cdot}CH_2R\ (R = Et, Ph)$$

$$\text{(XII.)}\ C_5H_{11}{\cdot}C{:}\overset{*}{C}{\cdot}CMe(OH){\cdot}CH_2Cl \longrightarrow Me{\cdot}CO{\cdot}CH_2{\cdot}C{:}C{\cdot}C_5H_{11}$$

(* = migrating group).

were proved either by comparison with products obtained by alternative methods, or by hydrogenation to known saturated ketones. From the examples given, it can readily be deduced that in this hydrobenzoin type of rearrangement the migratory ability of the various groups is in the order $Ph{\cdot}C{:}C > Ph, Et > C_5H_{11}{\cdot}C{:}C > Me$.

Reactions of Vinylacetylenic Carbinols.—I. N. Nazarov and his associates have made a detailed study of the reactions of vinylacetylenylcarbinols of the type (I), which they obtain in excellent yields by interaction of vinylacetylene with ketones in ethereal solution in the presence of potassium

$$\text{(I.)}\ CH_2{:}CH{\cdot}C{:}C{\cdot}C(OH)Me_2 \xrightarrow[60\text{–}70°]{Ac_2O\text{-trace }H_2SO_4} CH_2{:}CH{\cdot}C{:}C{\cdot}CMe{:}CH_2\ \text{(II.)}$$

hydroxide.[120] The Grignard method is stated [175] to be the only successful

[173] *Bull. Soc. chim.*, 1938, **5**, 1638; 1934, **1**, 1374; 1933, **53**, 1107; *Compt. rend.*, 1938, **206**, 1773; 1934, **198**, 183.

[174] *Compt. rend.*, 1941, **213**, 753; 1942, **214**, 892.

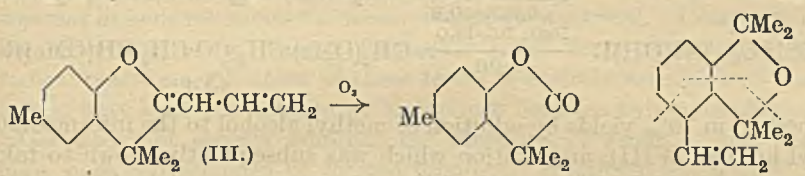
[175] I. N. Nazarov and A. N. Elizarova, *Bull. Acad. Sci. U.R.S.S.*, 1940, 189.

route to the primary and secondary carbinols, but recently it has been shown [176] that the latter can be prepared satisfactorily from sodio-vinylacetylenes in liquid ammonia. The tertiary carbinols polymerise with great facility,[120, 177] they can be dehydrated [31] to divinylacetylene homologues (*e.g.*, II) and with alcohols and sulphuric acid they give ethers, which polymerise somewhat more slowly than the carbinols. The etherification reaction has been shown to be reversible.[178]

Both dimethyl-vinylethynylcarbinol (I) and its dehydration product (vinyl*iso*propenylacetylene) in the presence of phosphoric acid condense readily with phenols or phenolic ethers in the *p*-position.[179] When only

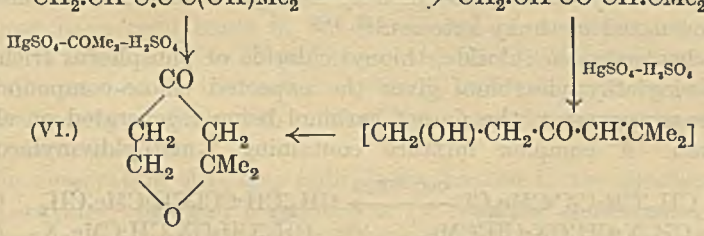
(I.) $CH_2{:}CH{\cdot}C{:}C{\cdot}C(OH)Me_2 \xrightarrow[H_3PO_4]{PhOH} CH_2{:}CH{\cdot}C{:}C{\cdot}CMe_2{\cdot}C_6H_4{\cdot}OH(p)$

o-substitution is possible, simultaneous cyclisation ensues, yielding the allylidenecoumarone derivative (III), its structure being proved by oxidation. The polymerisable vinyl*iso*coumarone (IV) is formed [180] in 70—80% yield



when the carbinol (I) is treated with formic or phosphoric acid, or with ferric chloride in benzene, the mechanism visualised comprising bimolecular dehydration to the ether, followed by an internal Diels–Alder addition reaction.

The reactions of vinylacetylenylcarbinols with mercuric salts are more complex than those of the simple ethynylcarbinols, but under carefully chosen conditions Nazarov has succeeded in isolating several interesting products. Under anhydrous conditions and apparently as a consequence of a Meyer–Schuster type of rearrangement the carbinol (I) is isomerised to vinyl *iso*butenyl ketone (V), but in the presence of dilute acids the unsatur-



ated ketone, presumably after an initial hydration, undergoes cyclisation to the tetrahydro-γ-pyrone (VI); good yields of either (V) or (VI) are obtained

[176] I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J.*, 1944, 140.
[177] W. H. Carothers and R. A. Jacobson, *J. Amer. Chem. Soc.*, 1933, **55**, 1097.
[178] I. N. Nazarov, *Bull. Acad. Sci. U.R.S.S.*, 1938, 706, 719, 726.
[179] *Idem et al., ibid.*, 1941, 431, 545. [180] *Idem, ibid.*, 1941, 556.

under the appropriate conditions.[120, 181] The homologues of (I), prepared from diethyl and methyl propyl ketones, behave differently, the predominating reaction under the above and related conditions being dehydration to the dienynes.[120] A similar difference in behaviour has been observed between the acetylenic glycols derived from acetone and from diethyl ketone (p. 173). The corresponding secondary carbinols are readily converted by the above procedure into monoalkyl tetrahydro-γ-pyrones related to (VI); also the formation of these pyrone derivatives from substituted divinyl ketones under the influence of acids and mercuric salts or of hydrogen chloride has been shown to be a general reaction.[181]

When any of the tertiary carbinols (VII) react with mercuric sulphate in methyl-alcoholic solution the unsaturated methoxy-ketones (IX) are

$$\underset{\text{(VII.)}}{CH_2{:}CH{\cdot}C{:}C{\cdot}C(OH)R_1R_2} \xrightarrow[HgO;\ BF_3\text{-}Et_2O;\ MeOH]{HgSO_4\text{-}MeOH\text{ or}} [CH_2{:}CH{\cdot}CO{\cdot}CH{:}CR_1R_2] \text{ (VIII.)}$$

$$\xrightarrow[p\text{-}C_6H_4Me\text{-}SO_3H]{} CH_2(OMe){\cdot}CH_2{\cdot}CO{\cdot}CH{:}CR_1R_2 \text{ (IX.)}$$

$$CH_2{:}CH{\cdot}C{:}C{\cdot}CH(OH)R \xrightarrow[MeOH]{HgO;\ BF_3\text{-}Et_2O} CH_2(OMe){\cdot}CH_2{\cdot}CO{\cdot}CH_2{\cdot}CH(OMe)R$$

(X.)

obtained [182] in 70% yields by addition of methyl alcohol to the intermediate divinyl ketones (VIII), an addition which was subsequently shown to take place very readily.[183] The reaction follows the same route when the mercuric oxide–boron trifluoride catalyst is employed, the structures of the resulting keto-ethers (IX) being demonstrated by the isolation of β-methoxypropionic acid and the expected ketones on ozonolysis.[184] Alcohols apparently add more readily to the less substituted ethenoid linkages in these unsaturated ketones, as already indicated above by the preferential reaction of methyl alcohol with the vinyl group, since reactions between the secondary carbinols and methyl alcohol with the mercury–boron trifluoride catalyst result in the production of saturated dimethoxy-ketones (X).[185]

Distillation of the methoxy-ketones (IX) from a trace of toluene-*p*-sulphonic acid yields the divinyl ketones (VIII), which readily polymerise, as also do the vinyl ketones obtained by elimination of methyl alcohol from the hydrogenated methoxy-ketones.[182, 186]

With dry hydrogen chloride, thionyl chloride or phosphorus trichloride, dimethyl-vinylethynylcarbinol gives the expected chloro-compound (XI) without rearrangement, the parent carbinol being regenerated on shaking with alkali. A complex mixture containing 2-methyldivinylacetylene

$$\underset{\text{(XI.)}}{CH_2{:}CH{\cdot}C{:}C{\cdot}CMe_2Cl} \xrightarrow{CuCl\text{-}NH_4Cl} CH_2{:}CH{\cdot}CCl{:}CH{\cdot}CMe{:}CH_2 \text{ (XII.)}$$

$$\underset{\text{(XIII.)}}{CH_2X{\cdot}CH{:}CX{\cdot}CH{:}CMe_2} \qquad CH_2{:}CH{\cdot}CX{:}CH{\cdot}CMe_2X \text{ (XIV.)}$$

and some (XII), which readily polymerises on keeping, results from the isomerisation of (XI) in contact with cuprous and ammonium chlorides

[181] I. N. Nazarov, *Bull. Acad. Sci. U.R.S.S.*, 1941, 423; 1943, 50.
[182] *Idem, ibid.*, 1940, 545. [183] *Idem, ibid.*, 1943, 206. [184] *Idem, ibid.*, 1940, 453.
[185] *Idem, ibid.*, 1941, 314. [186] *Idem, ibid.*, 1940, 552.

(cf. p. 170). On prolonged treatment of the carbinol with concentrated aqueous halogen acid at the ordinary temperature a difficultly separable mixture of two di-halogeno-compounds, formulated as (XIII) and (XIV), is obtained. The former is converted into a triene (*e.g.*, XII) on heating with water.[187] E. R. H. J.

6. Homolytic Reactions.

Introduction.

The majority of the reactions of organic chemistry are either reactions between molecules and ions or reactions between two molecules. In many cases it may be difficult to decide between these alternatives and both are frequently termed ionic reactions. To these must be added a third type, the prevalence and importance of which are becoming more and more recognised, namely, reactions between atoms or free radicals and molecules. In the latter reactions a symmetrical fission of an electron pair is involved, whereas in ionic reactions the fission is non-symmetrical. Reactions involving such electrically neutral fragments have been termed non-ionic or radical reactions. Neither of these terms is entirely satisfactory, since the former may lead to confusion with reactions between two molecules, and the latter does not strictly embrace the reactions of atoms. Such reactions may more conveniently be termed homolytic reactions, the terms "homolysis" and "heterolysis" having been first suggested by C. K. Ingold [1] in 1938 to describe the symmetrical and non-symmetrical fission of a covalent bond, thus :

$$\underset{\text{Homolysis}}{A{:}B \longrightarrow A\cdot + \cdot B} \qquad \underset{\text{Heterolysis}}{A{:}B \longrightarrow A + {:}B}$$

Up to about ten years ago our knowledge of the chemistry of free radicals was confined almost exclusively to those of the triphenylmethyl type, which are stabilised by resonance, and to the simpler hydrocarbon free radicals which participate in certain gaseous reactions. Valuable as this knowledge was it had comparatively little bearing on general laboratory and technical processes occurring in solution, but within recent years important advances have been made in our knowledge of reactions involving free radicals in solution. The subject was reviewed in 1937 [2] and again in 1940 [3] and full references to the earlier work will be found in these publications. The present report deals with most of the major developments which have taken place since 1940.

The observation that free radicals are formed in the decomposition of acyl peroxides [4] has had a number of far-reaching consequences. It has provided an explanation of the "peroxide effect"; [5, 6] secondly, it has

[187] I. N. Nazarov *Bull. Acad. Sci. U.R.S.S.*, 1942, 66, 135; 1943, 43.
[1] *Trans. Faraday Soc.*, 1938, **34**, 227.
[2] D. H. Hey and W. A. Waters, *Chem. Reviews*, 1937, **21**, 169.
[3] D. H. Hey, *Ann. Reports*, 1940, **37**, 250. [4] D. H. Hey, *J.*, 1934, 1966.
[5] M. S. Kharasch, H. Engelmann, and F. R. Mayo, *J. Org. Chem.*, 1937, **2**, 288.
[6] Ref. 2, p. 202.

supplemented Staudinger's theory of addition polymerisation to such purpose that very valuable extensions to our knowledge of the process have resulted; and thirdly, it has provided the basis for many substitution reactions involving chlorination, sulphonation, and carboxylation by means of chain reactions. As has been pointed out by F. R. Mayo and C. Walling,[7] a new and broader outlook on organic reactions in general has been opened up by the concept of chain reactions in solution, in which atoms or free radicals act as the chain carriers. Such a concept in many cases supersedes the earlier limited and inadequate notions which ascribed all reactions to simple unimolecular or bimolecular mechanisms. Further, reactions which involve the participation of atoms and free radicals frequently reveal many new and unexpected features, and compounds which are normally regarded as stable and inert often display remarkable reactivity. This new knowledge provides the chemist with novel and useful methods for use in synthesis and indicates the directions in which the search for new reactions of the so-called inert paraffin hydrocarbons of the petroleum industry should proceed. Reactions involving free radicals may also be of considerable importance in a number of biological processes.[8, 9] The mechanism and kinetics of reactions involving free radicals in solution have been discussed by W. A. Waters.[10]

Diazo and Related Compounds.

One of the most characteristic features of free aryl radicals is their capacity to react with aromatic compounds in such a way that the normal laws of aromatic substitution are not obeyed.[4, 11] This phenomenon, termed amphoteric or radical substitution, has been embraced within a general quantum mechanical treatment of the orientation of substituents in aromatic molecules which shows excellent qualitative agreement with the known facts.[12]

A comprehensive review of the usefulness for synthetic purposes of the Gomberg and the nitrosoacylarylamine reaction, which involve the participation of free radicals, has been published by W. E. Bachmann and R. A. Hoffmann.[13] Recent examples of the successful application of one or other of these methods include the synthesis of 1 : 2 : 5 : 6-dibenzfluorenone from *N*-nitrosoacet-2-naphthalide and amyl 2-naphthoate [14, 15] and the utilisation of the reaction between 3-*N*-nitrosoacetamido-4-cyanotoluene and various derivatives of quinol by R. Ghosh, D. S. C. Pascall, and A. R. Todd in their work on the constitution and synthesis of cannabinol.[16] The reaction between diazotised bases and pyridine has been used for the preparation

[7] *Chem. Reviews*, 1940, **27**, 351.
[8] W. A. Waters, *Trans. Faraday Soc.*, 1943, **39**, 140.
[9] *Idem, Chem. and Ind.*, 1944, 132.
[10] *Trans. Faraday Soc.*, 1941, **37**, 770.
[11] W. S. M. Grieve and D. H. Hey, *J.*, 1934, 1797.
[12] G. W. Wheland, *J. Amer. Chem. Soc.*, 1942, **64**, 900.
[13] "Organic Reactions," Vol. II, Chapter VI. Ed. Roger Adams, New York, 1944.
[14] G. Swain and A. R. Todd, *J.*, 1941, 674.
[15] A. R. Todd, G. Swain, and I.C.I. Ltd., B.P. 551,921.
[16] *J.*, 1940, 1118.

of a large number of pyridylquinolines of various types,[17, 18, 19] and a number of green pigments of the phthalocyanine series have been prepared from phenyl- and pyridyl-phthalonitrile and similar compounds, the phthalonitriles being prepared from diazotised 4-aminophthalonitrile and either benzene or pyridine.[20, 21] The same tetrapyridylphthalocyanine has also been obtained from the reaction between tetra-diazotised tetra-(4)-aminophthalocyanine and pyridine.[22] The preparation of biaryls by the interaction of 1-aryl-3 : 3-dialkyltriazens with various aromatic compounds has been further examined and extended.[23] These reactions are shown to conform to the general principles of radical substitution and in many cases provide good yields. The reactions take place in the presence of acids and the active agent is probably the covalent diazo-chloride : $ArN{:}N{\cdot}NMe_2 + 2HCl \longrightarrow ArN{:}NCl + NHMe_2,HCl$.

The action of diazonium compounds on unsaturated compounds has been investigated by H. Meerwein, E. Büchner, and K. van Emster,[24] who claimed that the aryl group replaces a hydrogen atom attached to an α-carbon atom with reference to a carbonyl group, and that in some cases the chlorine (from a diazonium chloride) becomes attached to the β-carbon atom. With cinnamic acid, carbon dioxide also is eliminated. Subsequently, C. F. Koelsch [25] studied similar reactions with methyl acrylate and acrylonitrile, and found that both compounds reacted readily with diazonium compounds but that in these cases the aryl group becomes attached to the β-carbon atom, thus :

$$ArN_2Cl + CH_2{:}CH{\cdot}CO_2Me \longrightarrow Ar{\cdot}CH_2{\cdot}CHCl{\cdot}CO_2Me + N_2$$
$$ArN_2Cl + CH_2{:}CH{\cdot}CN \longrightarrow Ar{\cdot}CH_2{\cdot}CHCl{\cdot}CN + N_2$$

These reactions, which show considerable promise as preparative methods, take place in presence of acetone, a cupric salt and a sodium acetate buffer. In a later publication [26] reactions were carried out with methyl crotonate, crotonic acid, methyl cinnamate and other unsaturated compounds and the results obtained showed that several of the constitutions originally assigned by Meerwein were incorrect. The final conclusions indicate that the volume occupied by the group at the β-carbon atom is not a factor which influences

[17] H. Coates, A. H. Cook, I. M. Heilbron, D. H. Hey, A. Lambert, and (in part) F. B. Lewis, *J.*, 1943, 401.

[18] A. H. Cook, I. M. Heilbron, D. H. Hey, A. Lambert, and (in part) A. Spinks, *ibid.*, p. 404.

[19] H. Coates, A. H. Cook, I. M. Heilbron, D. H. Hey, A. Lambert and (in part) F. B. Lewis, *ibid.*, p. 406.

[20] J. W. Haworth, I. M. Heilbron, D. H. Hey, R. Wilkinson and (the late) E. F. Bradbrook. In the press.

[21] E. F. Bradbrook, J. W. Haworth, I. M. Heilbron, D. H. Hey, and I.C.I. Ltd., U.S.P. 2,277,629.

[22] N. H. Haddock and I.C.I. Ltd., B.P. 530,881.

[23] J. Elks and D. H. Hey, *J.*, 1943, 441.

[24] *J. pr. Chem.*, 1939, **152**, 237.

[25] C. F. Koelsch, *J. Amer. Chem. Soc.*, 1943, **65**, 57.

[26] C. F. Koelsch and V. Boekelheide, *ibid.*, 1944, **66**, 412.

the position of entry of the aryl group and a mechanism involving free aryl radicals is suggested for these reactions, which may be represented as follows :

(a) $PhN_2^+ + Me{\cdot}CO{\cdot}O^- \rightleftharpoons PhN{:}N{\cdot}O{\cdot}COMe \longrightarrow Ph{\cdot} + Me{\cdot}CO{\cdot}O{\cdot} + N_2$
(b) $Ph{\cdot} + R{\cdot}CH{:}CHR' \longrightarrow CHPhR{\cdot}CHR'{\cdot}$
(c) $CHPhR{\cdot}CHR'{\cdot} + Cu^{++} \longrightarrow Cu^+ + CHPhR{\cdot}\overset{+}{C}HR'$
(d) $Cu^+ + Me{\cdot}CO{\cdot}O{\cdot} \longrightarrow Cu^{++} + Me{\cdot}CO{\cdot}O^-$
(e) $CHPhR{\cdot}\overset{+}{C}HR' + Cl^- \longrightarrow CHPhR{\cdot}CHClR'$
(f) $CHPhR{\cdot}\overset{+}{C}HR' \longrightarrow CPhR{:}CHR' + H^+$

The course followed in reaction (b) leads to the formation of the more stable of the two possible addition compounds.

C. E. Waring and J. R. Abrams [27] have reported some measurements on the thermal decomposition of benzenediazonium chloride in various solvents. This work is an extension of the earlier work of H. A. H. Pray [28] and, in agreement with previous results, the rates of decomposition were found to be of the first order in all the solvents investigated. The authors put forward a general mechanism to account for these results, involving the liberation of free radicals and their subsequent reaction with the solvent, which is substantially identical with that previously put forward by W. S. M. Grieve and D. H. Hey [11] to account for the results of H. A. H. Pray and for similar observations made with nitrosoacylarylamines.

Electrolytic Reactions.

Free radicals may be generated by the discharge of an ion at an electrode and results of particular interest have emerged from a study of the electrolysis of Grignard reagents in ethereal solution. The process may be represented thus :

$$(RMgX)_2 \longrightarrow Mg^{++} + (R_2MgX_2)^{--}$$

at the anode : $(R_2MgX_2)^{--} \longrightarrow 2R{\cdot} + MgX_2 + 2e$

at the cathode : $Mg^{++} + 2e \longrightarrow Mg$

The free radicals liberated at the anode may react (a) by dimerisation, (b) by disproportionation, and (c) by removing hydrogen from the solvent (ether). The participation of the ether in these reactions was confirmed by the electrolysis of methylmagnesium iodide in *n*-butyl ether.[29] The products, in addition to methane, consisted of butane, butylene, methylpropylcarbinol and *n*-butyl alcohol. The formation of these products clearly indicates that the fourth hydrogen atom in the methane is provided by the solvent. The free methyl radical abstracts hydrogen attached to both the

[27] *J. Amer. Chem. Soc.*, 1941, **63**, 2757.
[28] *J. Physical Chem.*, 1926, **30**, 1477.
[29] W. V. Evans and E. Field, *J. Amer. Chem. Soc.*, 1936, **58**, 720, 2284.

α- and the β-carbon atom of the ether but not apparently from the γ- and the δ-position. These reactions may be represented as follows :

$$\text{(a) Me}\cdot + n\text{-BuO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_3 \longrightarrow \text{CH}_4 + n\text{-BuO}\cdot\dot{\text{C}}\text{H}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_3$$

$$n\text{-BuO}\cdot\dot{\text{C}}\text{H}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_3 \longrightarrow n\text{-Bu}\cdot + \text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CHO}$$

$$\downarrow \qquad\qquad \downarrow \text{MeMgI}$$

$$\text{C}_4\text{H}_8 + \text{C}_4\text{H}_{10} \quad \text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH(OH)}\cdot\text{CH}_3$$

$$\text{(b) Me}\cdot + n\text{-BuO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_3 \longrightarrow \text{CH}_4 + n\text{-BuO}\cdot\text{CH}_2\cdot\dot{\text{C}}\text{H}\cdot\text{CH}_2\cdot\text{CH}_3$$

$$n\text{-BuO}\cdot\text{CH}_2\cdot\dot{\text{C}}\text{H}\cdot\text{CH}_2\cdot\text{CH}_3 \longrightarrow n\text{-BuO}\cdot + \text{CH}_2\text{:CH}\cdot\text{CH}_2\cdot\text{CH}_3$$

$$\downarrow$$

$$n\text{-BuO}\cdot\text{MgI} \longrightarrow n\text{-BuOH}$$

Similar results were obtained in the electrolysis of *n*-propylmagnesium bromide and *iso*propylmagnesium bromide in ethereal solution.[30] As the length of the chain increases, the tendency for the free radicals to dimerise increases and reactions carried out with *n*-butyl-, *iso*butyl-, *sec.*-butyl-, *tert.*-butyl-, and *n*-hexyl-magnesium bromide showed that with the higher radicals the reactions with the solvent become negligible.[31]

W. V. Evans, R. Pearson, and D. Braithwaite [32] investigated the electrolysis of some aromatic Grignard reagents. The results are summarised below :

Grignard Reagent.	*Main Products.*
Phenylmagnesium bromide	Benzene, diphenyl, *p*-terphenyl, styrene, higher hydrocarbons
p-Tolylmagnesium bromide	*p*-Methylstyrene, higher hydrocarbons; no ditolyl
p-Chlorophenylmagnesium bromide	*p*-Chlorostyrene, higher hydrocarbons; no 4 : 4′-dichlorodiphenyl
Benzylmagnesium bromide	Dibenzyl in high yield, some higher hydrocarbons

From these results the authors concluded that, in contrast to the aliphatic free radicals, the chief characteristic of free aryl radicals is neither dimerisation nor disproportionation but reaction with the solvent, in agreement with the earlier conclusions of D. H. Hey and W. A. Waters.[2] The main reactions may be represented thus :

$$\text{Ph}\cdot + \text{CH}_3\cdot\text{CH}_2\cdot\text{OEt} \longrightarrow \text{PhH} + \text{CH}_3\cdot\dot{\text{C}}\text{H}\cdot\text{OEt}$$

$$\text{CH}_3\cdot\dot{\text{C}}\text{H}\cdot\text{OEt} \longrightarrow \text{Et}\cdot + \text{CH}_3\cdot\text{CHO}$$

$$2\text{Et}\cdot \longrightarrow \text{C}_2\text{H}_4 + \text{C}_2\text{H}_6$$

$$\text{CH}_3\cdot\text{CHO} + \text{PhMgBr} \longrightarrow \text{CH}_3\cdot\text{CH(OH)Ph} \longrightarrow \text{CH}_2\text{:CHPh}$$

The authors also considered that some phenyl radicals undergo dimerisation

[30] W. V. Evans and D. Braithwaite, *J. Amer. Chem. Soc.*, 1939, **61**, 898.
[31] W. V. Evans, D. Braithwaite, and E. Field, *ibid.*, 1940, **62**, 534.
[32] *Ibid.*, 1941, **63**, 2574.

at the anode surface, where the concentration of free radicals is greatest, to give diphenyl, which in turn may give higher hydrocarbons thus :

$$2Ph\cdot \longrightarrow Ph_2$$
$$Ph\cdot + Ph_2 \longrightarrow PhH + Ph\cdot C_6H_4\cdot$$
$$PhC_6H_4\cdot + Ph\cdot \longrightarrow Ph\cdot C_6H_4Ph, \text{ etc.}$$

It seems highly probable that these hydrocarbons also result from reactions between the aryl radicals and benzene or diphenyl molecules,[11] thus :

$$Ph\cdot + C_6H_6 \longrightarrow Ph_2 + H\cdot$$
$$Ph\cdot + Ph_2 \longrightarrow Ph\cdot C_6H_4Ph + H\cdot, \text{ etc.}$$

The authors interpret the vigorous attack on hydrogen-bearing solvents by free aryl radicals as an indication that aryl radicals are more reactive than simple aliphatic radicals.

Halogenation, Sulphonation, and Carboxylation.

The discovery of the capacity of acyl peroxides to yield free radicals on thermal decomposition [4] has led to the extensive utilisation of benzoyl peroxide to initiate chain reactions involving free radicals and atoms. Reactions of this type have been studied by M. S. Kharasch and his co-workers, who have developed therefrom novel methods of chlorination, bromination, sulphonation, and carboxylation. These results are of considerable importance for preparative purposes, especially since the products obtained as the result of a homolytic process are frequently different from those obtained by substitution processes involving ions and molecules. H. C. Brown, M. S. Kharasch, and T. H. Chao [33] have drawn attention to the increasing number of reactions which involve an atomic or free radical mechanism. These include not only peroxide-catalysed chlorination with sulphuryl chloride, photochemical sulphonation with sulphuryl chloride, and peroxide-catalysed and photochemical carboxylation with oxalyl chloride, but also the addition reactions of hydrogen bromide, thiols, and sodium bisulphite with olefins in presence of peroxides.

M. S. Kharasch and his co-workers have developed the use of sulphuryl chloride for both chlorination and sulphonation reactions.[34] It has been shown that, whereas sulphuryl chloride normally furnishes chlorine molecules, in presence of peroxides chlorine atoms are liberated.[35] By the use of sulphuryl chloride in presence of benzoyl peroxide it is thus possible to chlorinate in the dark many types of compound (*e.g.*, paraffins, aromatic side chains, acids, etc.) far more rapidly and conveniently than is possible by the use of chlorine in sun-light. The reactive intermediate is the chlorine

[33] *J. Amer. Chem. Soc.*, 1940, **62**, 3435.
[34] H. C. Brown, *Ind. Eng. Chem.*, 1944, **36**, 785.
[35] M. S. Kharasch and H. C. Brown, *J. Amer. Chem. Soc.*, 1939, **61**, 2142.

atom, as in photochemical chlorination, and the following mechanism, involving the free radical R·, is suggested :

$$(Ph{\cdot}CO{\cdot}O)_2 \longrightarrow Ph{\cdot} + Ph{\cdot}CO{\cdot}O{\cdot} + CO_2$$
$$Ph{\cdot}CO{\cdot}O{\cdot} \longrightarrow Ph{\cdot} + CO_2$$
$$Ph{\cdot} + SO_2Cl_2 \longrightarrow {\cdot}SO_2Cl + PhCl$$
$${\cdot}SO_2Cl \longrightarrow SO_2 + Cl{\cdot}$$
$$Cl{\cdot} + RH \longrightarrow R{\cdot} + HCl$$
$$R{\cdot} + SO_2Cl_2 \longrightarrow RCl + {\cdot}SO_2Cl$$

Sulphuryl chloride does not react with aliphatic acids and acid chlorides in the dark or in the absence of a catalyst but only if reaction is initiated with a peroxide.[36] Acetic acid and acetyl chloride are exceptions. With propionic acid both the α- and the β-chloro-compound are formed, the latter predominating, and in all cases investigated substitution takes place preferentially on the carbon atom some distance removed from the carboxyl group. The results obtained with a number of acids and acid chlorides were as follows :

Acid or Acid Chloride.	% Yield.			Acid or Acid Chloride.	% Yield.		
	α.	β.	γ.		α.	β.	γ.
Propionic acid.....	45	55	—	*iso*Butyryl chloride ...	20	80	—
Propionyl chloride	40	60	—	*n*-Butyric acid	10	45	45
*iso*Butyric acid	15	85	—	*n*-Butyryl chloride ...	15	55	30

Other catalysts, such as iodine, do not give these abnormal results, but similar results had been previously reported as the result of photochemical chlorination. Both the photochemical chlorination and the peroxide-catalysed reaction with sulphuryl chloride proceed by means of a chain reaction involving chlorine atoms.

In the presence of light sulphuryl chloride acts as a sulphonating agent towards acids and again β- and γ-substitution take place instead of the customary α-sulphonation, such as occurs with sulphur trioxide or chlorosulphonic acid.[36] A free-radical mechanism is proposed for the β- and γ-sulphonation, as opposed to the ionic mechanism of α-substitution. M. S. Kharasch and (Miss) A. T. Reid [37] also found that pyridine or quinoline in presence of light catalyses the sulphonation of aliphatic hydrocarbons by means of sulphuryl chloride. These reactions are again attributed to free radicals, as represented below :

$$\text{(a)}\ SO_2Cl_2 \xrightarrow{Py} SO_2 + Cl_2$$
$$\text{(b)}\ Cl_2 + h\nu \longrightarrow 2Cl{\cdot}$$
$$\text{(c)}\ Cl{\cdot} + RH \longrightarrow R{\cdot} + HCl$$
$$\text{(d)}\ R{\cdot} + SO_2 \longrightarrow R{\cdot}SO_2{\cdot}$$
$$\text{(e)}\ R{\cdot}SO_2{\cdot} + Cl_2 \longrightarrow R{\cdot}SO_2Cl + Cl{\cdot}$$

The fundamental reaction which leads to sulphonation is stage (a), which

[36] M. S. Kharasch and H. C. Brown, *J. Amer. Chem. Soc.*, 1940, **62**, 925.
[37] *Ibid.*, 1939, **61**, 3089.

gives free chlorine molecules in solution. In the peroxide-catalysed chlorination reaction (a) is not involved.[38]

In a study of the photolysis of both carbonyl chloride and oxalyl chloride in *cyclo*hexane, M. S. Kharasch and H. C. Brown [39] showed that the acid chloride of *cyclo*hexanecarboxylic acid is formed in quantitative yield. The reaction can also be initiated by peroxides in place of light. The same authors extended these reactions to various paraffin hydrocarbons.[40] In the dark and in absence of peroxides no reaction takes place between oxalyl chloride and *n*-heptane or *cyclo*hexane even when boiling. In the presence of light, or in the dark in presence of benzoyl peroxide, reaction readily occurs at room temperature to give an acid chloride. The mechanisms proposed for these reactions may be represented as follows :

Primary process in light : $(COCl)_2 + h\nu \longrightarrow 2\cdot COCl$

and/or $(COCl)_2 + h\nu \longrightarrow \cdot CO\cdot COCl + Cl\cdot$

Primary process with peroxide : $(Ph\cdot CO\cdot O)_2 \longrightarrow Ph\cdot + Ph\cdot CO\cdot O\cdot + CO_2$

$Ph\cdot + (COCl)_2 \longrightarrow Ph\cdot COCl + \cdot COCl$

Secondary process : $\cdot COCl \longrightarrow CO + Cl\cdot$

$\cdot CO\cdot COCl \longrightarrow 2CO + Cl\cdot$

$RH + Cl\cdot \longrightarrow R\cdot + HCl$

$R\cdot + (COCl)_2 \longrightarrow R\cdot COCl + \cdot COCl$

Acyl Peroxides.

H. Wieland and A. Meyer [41] have examined the action of benzoyl peroxide on triphenylmethyl in benzene solution. Tetraphenylmethane is formed in small yield and evidence is obtained which proves that the fourth phenyl group in this hydrocarbon is derived not from the peroxide but from the solvent. These results may be represented as follows :

$(Ar\cdot CO\cdot O)_2 \longrightarrow 2Ar\cdot CO\cdot O\cdot$

$Ar\cdot CO\cdot O\cdot + RH \longrightarrow Ar\cdot CO\cdot OH + R\cdot$

$R\cdot + \cdot CPh_3 \longrightarrow R\cdot CPh_3$

In a study of the chlorination of primary active amyl chloride [33] (both peroxide-catalysed and photochemical) it was shown that inactive 1 : 2-dichloro-2-methylbutane is formed, which supports mechanism A, involving an alkyl radical, rather than mechanism B :

(A) $Cl\cdot + RH \longrightarrow R\cdot + HCl$

$R\cdot + Cl_2 \longrightarrow RCl + Cl\cdot$

(B) $Cl\cdot + RH \longrightarrow RCl + H\cdot$

$H\cdot + Cl_2 \longrightarrow HCl + Cl\cdot$

[38] M. S. Kharasch, T. H. Chao, and H. C. Brown, *J. Amer. Chem. Soc.*, 1940, **62**, 2393.

[39] *Ibid.*, p. 454.

[40] *Ibid.*, 1942, **64**, 329.

[41] *Annalen*, 1942, **551**, 249.

In the latter case the product would be expected to retain optical activity. As pointed out by M. S. Karasch, S. S. Kane, and H. C. Brown,[42] the assumption is made that no rearrangement of the free radical occurs in this process. On this assumption the proportions of *n*-propyl and *iso*propyl chlorides obtained in the chlorination of propane should be the same as the proportions of the corresponding free radicals first formed. In order to test this principle these authors investigated the decomposition of *n*-butyryl and *iso*butyryl peroxide in carbon tetrachloride solution. Under these conditions the following reactions occur :

$$(R{\cdot}CO{\cdot}O)_2 \longrightarrow R{\cdot} + R{\cdot}CO{\cdot}O{\cdot} + CO_2$$
$$R{\cdot} + CCl_4 \longrightarrow RCl + {\cdot}CCl_3$$
$$2{\cdot}CCl_3 \longrightarrow C_2Cl_6$$

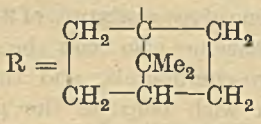
Decomposition of *n*-butyryl peroxide gave only *n*-propyl chloride and no *iso*propyl chloride; similarly, *iso*butyryl peroxide gave *iso*propyl chloride and no trace of the normal isomeride. It is therefore concluded that no isomerisation of the free radicals takes place in solution in these reactions.

In order to gain further insight into the relative activity of free radicals M. S. Kharasch, S. S. Kane, and H. C. Brown [43] studied the action of various peroxides on carbon tetrachloride. Whereas methyl chloride, *n*-propyl chloride, and *iso*propyl chloride were readily isolated from the reactions with acetyl, butyryl, and *iso*butyryl peroxides, no *tert.*-butyl chloride or benzyl chloride could be found in the corrresponding reactions with trimethylacetyl and phenylacetyl peroxides. Benzoyl peroxide and carbon tetrachloride readily give chlorobenzene. It is therefore inferred that the methyl, propyl, *iso*propyl, and phenyl radicals are more reactive than the *tert.*-butyl and the benzyl radical.

Since free radicals of the type $R_1R_2R_3C{\cdot}$ yield optically inactive products $R_1R_2R_3CX$, whether the radical is of short or long life, it is assumed that a coplanar configuration is attained in at least one phase of its internal vibrations. Certain radicals, however, cannot assume a coplanar configuration. Such a radical is the 1-apocamphyl radical, which may be generated in solution from the peroxide of apocamphane-1-carboxylic acid. The reaction of this peroxide with carbon tetrachloride was studied by M. S. Kharasch, F. Engelmann, and W. H. Urry,[44] and the following products in the quantities indicated were isolated :

$$(R{\cdot}CO{\cdot}O)_2 \xrightarrow{CCl_4} \underset{36\%}{RCl} + \underset{9\%}{R_2} + \underset{50\%}{R{\cdot}CO{\cdot}OR} + \underset{5\%}{R{\cdot}CO{\cdot}OH} + C_2Cl_6$$

where



[42] *J. Amer. Chem. Soc.*, 1941, **63**, 526.
[43] *Ibid.*, 1942, **64**, 1621.
[44] *Ibid.*, 1943, **65**, 2428.

The mechanism proposed by these authors to account for the various products may be represented as follows :

$$\begin{array}{ll}
\text{(a)} & (\text{R·CO·O})_2 \longrightarrow \text{R·} + \text{R·CO·O·} + \text{CO}_2 \\
\text{(b)} & \text{R·} + \text{CCl}_4 \longrightarrow \text{RCl} + \text{·CCl}_3 \\
\text{(c)} & \text{R·} + \text{R·CO·O·} \longrightarrow \text{R·CO·OR} \\
\text{(d)} & \text{R·} + (\text{R·CO·O})_2 \longrightarrow \text{R·CO·OR} + \text{R·CO·O·} \\
\text{(e)} & 2\text{R·} \longrightarrow \text{R}_2 \\
\text{(f)} & \text{R·} + (\text{R·CO·O})_2 \longrightarrow \text{R}_2 + \text{CO}_2 + \text{R·CO·O·} \\
\text{(g)} & \text{R·CO·O} \longrightarrow \text{R·} + \text{CO}_2 \\
\text{(h)} & 2\text{·CCl}_3 \longrightarrow \text{C}_2\text{Cl}_6
\end{array}$$

In view of the relative stability of the peroxide the concentration of the free radical R· at any one moment will be low, hence reactions (d) and (f) are probably more prominent than reactions (c) and (e). The factors which determine the nature and respective quantities of the products are primarily the stability of the peroxide, its concentration, and the nature of the solvent.

In an investigation of the action of acyl peroxides on aliphatic acids by M. S. Kharasch and M. T. Gladstone,[45] it is shown that the reaction between acetyl peroxide and glacial acetic acid at 95—100° leads to the formation of succinic acid, methane, and carbon dioxide. In similar manner *iso*-butyric acid and chloroacetic acid give tetramethylsuccinic acid and *meso*-dichlorosuccinic acid respectively. The initial decomposition of the acetyl peroxide is assumed to give both acetate and methyl radicals :

$$(\text{Me·CO·O})_2 \longrightarrow \text{Me·} + \text{Me·CO·O·} + \text{CO}_2$$

The mechanism suggested for the formation of the dicarboxylic acids in these reactions may be represented as follows :

$$\begin{array}{l}
\text{Me·} + \text{Me·CO·OH} \longrightarrow \text{CH}_4 + \text{·CH}_2\text{·CO·OH} \\
2\text{·CH}_2\text{·CO·OH} \longrightarrow (\text{CH}_2\text{·CO·OH})_2
\end{array}$$

It is assumed that only the free methyl radicals have sufficient energy to remove the α-hydrogen atoms from the acid, and the dimerisation of the ·CH_2·CO·OH radical is to be expected, since the radical would probably be stabilised by resonance and would require a high energy of activation to react with the solvent.

L. F. Fieser, R. C. Clapp, and W. H. Daudt [46] have reported the methylation of aromatic nitro-compounds with lead tetra-acetate. With trinitrotoluene, trinitro-*m*-xylene is formed in a reaction in which carbon dioxide is evolved and a large excess of lead tetra-acetate is consumed. In similar manner 1 : 3 : 5-trinitrobenzene gives trinitrotoluene and trinitro-*m*-xylene, and *m*-dinitrobenzene gives a mixture of 2 : 4-dinitrotoluene and 2 : 4-dinitro-*m*-xylene. Nitrobenzene also can be methylated, though less readily than the polynitro-compounds, and the products are *o*- and *p*-nitrotoluene. In reaction with benzene the product is benzyl acetate, which implies methylation, followed by side-chain acetoxylation. These

[45] *J. Amer. Chem. Soc.*, 1943, **63**, 15. [46] *Ibid.*, 1942, **64**, 2052.

reactions show a number of peculiar features. In most cases a marked induction period is involved and compounds containing active hydrogen atoms act as promoters. In many cases reaction can also be initiated by heating. The fact that nuclear methylation takes place at the *o*- and *p*-positions with respect to both NO_2 and Cl, coupled with the increased reactivity of the polynitro-compounds, seems to indicate that the lead tetra-acetate breaks down with the liberation of free methyl radicals, possibly through the intermediate formation of acetyl peroxide, thus :

$$(Me{\cdot}CO{\cdot}O)_4Pb \longrightarrow (Me{\cdot}CO{\cdot}O)_2 + (Me{\cdot}CO{\cdot}O)_2Pb$$
$$(Me{\cdot}CO{\cdot}O)_2 \longrightarrow Me{\cdot} + Me{\cdot}CO{\cdot}O{\cdot} + CO_2$$

This interpretation is supported by the fact that trinitrotoluene may be converted into trinitro-*m*-xylene both by the action of acetyl peroxide in acetic acid solution and by electrolysis in acetic acid solution saturated with sodium acetate. L. F. Fieser and F. C. Chang [47] have also shown that quinones may be methylated by means of lead tetra-acetate.

L. F. Fieser and A. E. Oxford [48,49] have provided many examples of the use of acyl peroxides, including those of higher fatty acids, for the alkylation of quinones. In these reactions no promoter is necessary and no induction period is involved, from which it would appear that these phenomena are associated not with the actual methylation process but rather with the initial breakdown of the lead tetra-acetate.

N-*Bromosuccinimide.*

The use of *N*-bromosuccinimide for effecting substitution in olefins at the α-methylene or allyl position has recently been reported [50, 51] and it seems likely that these reactions involve bromine atoms. As W. A. Waters has recently pointed out,[52] halogen substitution in the α-methylenic position to a double bond is to be expected if the reaction is of the free-radical or atomic type, as indicated recently by E. H. Farmer [53] and by G. F. Bloomfield.[54] The atomic chlorination of *cyclo*hexene to yield Δ^2-*cyclo*hexenyl chloride was reported in 1939 by W. A. Waters,[55] who used benzenediazonium chloride as the source of the chlorine atoms. W. A. Waters points out [52] that reaction mechanisms involving the neutral bond fission of " positive halogen " compounds, such as the *N*-halogenoimides, should be applied with caution, since chemical changes of this type are in general chain processes which are very dependent on the concentrations and energy levels of the transient radicals as well as on the non-polar character of the solvent.

A particularly interesting example of the use of *N*-bromosuccinimide is its application to the degradation of the C_{17} side chain in bile acids and similar compounds.[56] Such acids can be degraded in stages by the method

[47] *J. Amer. Chem. Soc.*, 1942, **64**, 2043. [48] *Ibid.*, p. 2060.
[49] *J.*, 1942, 577, 583. [50] K. Ziegler *et al.*, *Annalen*, 1942, **551**, 80.
[51] F. S. Spring, *Ann. Reports*, 1943, **40**, 104.
[52] *Nature*, 1944, **154**, 772. [53] *Trans. Faraday Soc.*, 1942, **38**, 340.
[54] *J.*, 1944, 114. [55] *J.*, 1939, 1805.
[56] Society of Chemical Industry in Basle, B.P. Application 13,438, 1943.

of Wieland, but only one carbon atom is eliminated at each treatment. By the use of *N*-bromosuccinimide three carbon atoms in the side chain can be removed in one treatment, and the application of this series of reactions to the methyl ester of 3-hydroxycholenic acid (with appropriate protection of the nuclear double bond and final oxidation of the 3-hydroxyl group to the 3-keto-group) affords a new route to progesterone. The reaction at the C_{17} side chain may be represented thus :

$$R\cdot CHMe\cdot CH_2\cdot CH_2\cdot CO_2Me \xrightarrow{MeMgI} R\cdot CHMe\cdot CH_2\cdot CH{:}CMe_2 \longrightarrow$$
$$R\cdot CHMe\cdot CHBr\cdot CH{:}CMe_2 \longrightarrow R\cdot CMe{:}CH\cdot CH{:}CMe_2 \longrightarrow R\cdot COMe$$

Addition Polymerisation.

The theory of the mechanism of addition polymerisation, first put forward by H. Staudinger [57] in 1936, involves the primary formation of an activated molecule, which then reacts with a second molecule to give an activated dimeride; this in turn reacts successively with further molecules in the same way until finally some unknown factor causes the active centres to disappear. The initial activated unit and the intermediate stages in the building up of the polymer are highly reactive free radicals incapable of isolation. Activation can be brought about by the action of heat, light or free radicals and it is now recognised that the final deactivation may be the result of disproportionation or the incorporation in the polymer of foreign groups derived from the catalyst, the solvent, or from impurities. That the polymerisation of vinyl compounds involves a free-radical chain reaction, which is initiated by radicals formed in the thermal decomposition of the catalyst, is supported by a number of kinetic studies.[58, 59] The pre-eminent catalysts for effecting the polymerisation of vinyl compounds are peroxides, usually benzoyl peroxide.

The acceptance of this free-radical mechanism involves a number of corollaries. In the first place the molecules of the polymers so formed should contain fragments of the catalyst; secondly, if polymerisation is effected in presence of a solvent, the polymer should contain fragments of the solvent molecules; and, thirdly, compounds other than peroxides, which can give rise to free radicals, also should be capable of effecting polymerisation. Recent investigations have been directed to all three factors and the results obtained provide striking evidence in support of the free-radical theory.

The presence of fragments of the catalyst in the molecules of polymers is unmistakably revealed in a large number of investigations in which substituted or " marked " peroxides have been used. C. C. Price, R. W. Kell, and E. Krebs [60] have reported that, when styrene is polymerised by means of peroxides containing atoms or groups the presence of which can be readily

[57] *Trans. Faraday Soc.*, 1936, **32**, 97, 323.
[58] For references, see H. W. Melville, *Ann. Reports*, 1939, **36**, 61.
[59] C. C. Price and R. W. Kell, *J. Amer. Chem. Soc.*, 1941, **63**, 2798.
[60] *Ibid.*, 1942, **64**, 1103.

confirmed by analysis, *e.g.*, *p*-bromobenzoyl, *p*-chlorobenzoyl, or anisoyl peroxides, the polymer contains on an average from one half to two and a half groups derived from the peroxide in each molecule of the polymer. The polymerisation of styrene in the presence of *m*-bromobenzoyl peroxide has been studied by H. F. Pfann, D. J. Salley, and H. Mark [61] and again polymers containing bromine were obtained. In a later study of the polymerisation of styrene in the presence of 3 : 4 : 5-tribromobenzoyl peroxide C. C. Price and B. E. Tate [62] showed that the purified polystyrene contained 15% of bromine, which corresponds to approximately one tribromophenyl group for each molecule of polymer, and, with the use of improved methods, these authors concluded that, contrary to earlier findings, each molecule of polymer contained in general only one fragment of the catalyst. All polymers contained varying amounts of oxygen and references to the presence of oxygen in polystyrene have also been made elsewhere.[63, 64]

The significance of the presence of oxygen in polystyrene was investigated by P. D. Bartlett and S. G. Cohen,[65] who, using *p*-chloro- and *p*-bromobenzoyl peroxide as catalysts, showed that most of the halogen in the polymer exists in the form of the *p*-halogenobenzoate group, the remainder being present in the form of the *p*-halogenophenyl group, which cannot be removed by hydrolysis. These results are contrary to the earlier observations of C. C. Price, R. W. Kell, and E. Krebs,[60] who concluded that no ester groups were present, since no hydrolysis was effected on boiling with 20% aqueous potassium hydroxide. P. D. Bartlett and S. G. Cohen pointed out that the latter conditions are not sufficient to effect hydrolysis, which requires the use of sodium ethoxide in ethyl alcohol or a mixture of ethyl alcohol and toluene.

J. W. Breitenbach and G. Bremer [66] showed that, when the polymerisation of indene is effected with *o*- and *p*-chlorobenzoyl peroxide, the resultant polymers contain chlorine, and W. Kern and H. Kämmerer [63, 67] have utilised the participation of fragments of a " marked " catalyst in the polymer as the basis of a method for molecular-weight determination, which with certain assumptions gives good agreement with the values obtained from measurements of specific viscosity.

C. C. Price, R. W. Kell, and E. Krebs [60] have recorded the interesting observation that no polymerisation of styrene could be effected when a nitrobenzoyl peroxide was used and concluded that the presence of the nitro-group inhibits polymerisation. This observation is confirmed in a study of the relative efficiencies of some substituted benzoyl peroxides as catalysts for the polymerisation of methyl methacrylate.[68] This effect of the nitro-group is in keeping with earlier observations on the radical sub-

[61] *J. Amer. Chem. Soc.*, 1944, **66**, 983. [62] *Ibid.*, 1943, **65**, 517.
[63] W. Kern and H. Kämmerer, *J. pr. Chem.*, 1943, **161**, 289.
[64] C. C. Price, *J. Amer. Chem. Soc.*, 1943, **65**, 2380. [65] *Ibid.*, p. 543.
[66] *Ber.*, 1943, **76**, 1124. [67] *J. pr. Chem.*, 1942, **161**, 81.
[68] H. N. Alyea, J. J. Gartland, and H. R. Graham, *Ind. Eng. Chem.*, 1942, **34**, 458.

stitution of various aromatic compounds,[11] when it was shown that nitro-compounds reacted more readily with the free radicals than did other substituted hydrocarbons. Further observations on the retardation or inhibition of polymerisation by means of nitro-compounds have been made by C. C. Price and D. A. Durham,[69] who confirmed that this action was due to a direct reaction between the free radical and the nitro-compound. The preparation of polystyrene in presence of either nitrobenzene or 2 : 4-dinitrochlorobenzene gave products which contained nitro-groups. That the effect produced by the aromatic nitro-compound is due to the activation of the nuclei in the manner previously suggested and not to a direct effect of the nitro-group as such was proved by the fact that polymerisation of styrene in presence of nitromethane proceeded in a normal manner. C. C. Price [64] has also shown that nitrothiophen is more reactive in retarding polymerisation than is nitrobenzene and the polystyrene formed in the presence of the former contained one nitrothienyl group in each polymer chain. Polystyrene prepared in the presence of chloranil was found to contain chloranil, although in this case there was no retardation.

The incorporation of fragments of the catalyst in the polymer molecules is a matter of some importance in the chemistry of rubber. E. H. Farmer and S. E. Michael [53, 70] have shown that the action of benzoyl peroxide on rubber produces a species of vulcanisation. Other compounds which can give rise to free radicals should also produce similar results and it is therefore not surprising to find that B. V. Buizov [71] had already shown that certain diazo-compounds were active vulcanising agents for rubber.

S. Kamenskaya and S. Medvedev [72] have shown that solvents also can participate in reactions with the growing chains during polymerisation. Similar results have been reported by J. W. Breitenbach and A. Maschin,[73] who, using carbon tetrachloride as solvent, obtained polymers containing chlorine. When styrene is polymerised in presence of a solvent, the resulting polymer is of lower molecular weight than that obtained when polymerisation is effected in the absence of a solvent. F. R. Mayo [74] has attributed this to a chain transfer between the growing polymer and the solvent and has made a comparison of the effectiveness of various solvents in the chain transfer process.

Many examples have been recorded of the initiation of polymerisation by compounds other than peroxides, which can decompose to liberate free radicals. G. V. Schulz and G. Wittig [75] have demonstrated that the polymerisation of styrene can be initiated and accelerated by tetraphenylsuccinonitrile, and G. V. Schulz [76] has also shown that similar results can be obtained with benzeneazotriphenylmethane. C. C. Price and D. A. Durham [77]

[69] *J. Amer. Chem. Soc.*, 1943, **65**, 757.
[70] *J.*, 1942, 513.
[71] *J. Russ. Phys. Chem. Soc.*, 1921, **53**, 166.
[72] *Acta Physicochim. U.R.S.S.*, 1940, **13**, 565.
[73] *Z. phyiskal. Chem.*, 1940, *A*, **187**, 175.
[74] *J. Amer. Chem. Soc.*, 1943, **65**, 2324.
[75] *Naturwiss.*, 1939, **27**, 387.
[76] *Ibid.*, p. 659.
[77] *J. Amer. Chem. Soc.*, 1942, **64**, 2508.

have shown that *p*-bromobenzenediazonium hydroxide catalyses the polymerisation of styrene and by measurement of the viscosity of the polymer it was regarded as containing an average of about 22 units and showed a bromine content of 4·2%, which corresponds approximately to one *p*-bromophenyl group for each molecule of the polymer. A. T. Blomquist, J. K. Johnson, and H. J. Sykes [78] have demonstrated that *N*-nitrosoacylarylamines can act as catalysts for polymerisation. Experiments were carried out with styrene, methyl methacrylate, and acrylonitrile and in order to determine which radicals were incorporated in the molecule of the polymer nitrosoacylarylamines containing bromine in both the aryl and the acyl group in turn were used, *e.g.*, *N*-nitroso-*p*-bromoacetanilide and *NN'*-dinitroso-4 : 4'-dibromosuccindianilide, to give bromophenyl radicals, and *N*-nitroso-α-bromo*iso*valeranilide and *N*-nitroso-*m*-bromobenzanilide to give bromoacyloxy-radicals. In the former cases bromine was found in the polymer, but with the latter no bromine was found. It was therefore inferred that when nitrosoacylarylamines are used as catalysts in these reactions, only the free aryl radicals are incorporated in the polymer. Determination of the bromine content and molecular weight showed that fragments of the catalyst were incorporated in the polymer over a wide range of values. The four types of compound which were shown to give rise to radical substitution [4, 11] have thus now all been tested as catalysts for addition polymerisation and in each case a positive result has been obtained.

The thermal polymerisation of styrene in the absence of a catalyst is also considered by C. Walling [79] to involve a free-radical mechanism as a result of an investigation into the polymerisation of styrene in phenolic solvents. Under these conditions the rate of polymerisation was found to bear no simple relationship to the acid strength of the phenol. On the other hand, the mechanism of addition polymerisation with other types of catalyst such as boron fluoride, stannic chloride, or aluminium chloride, is obviously of a different character.[80]

Grignard Reactions.

It has long been suspected that certain of the less familiar reactions of Grignard reagents may involve the participation of free radicals. The action of ethylmagnesium bromide on ethyl bromide gives not only *n*-butane but also ethane and ethylene and the formation of the latter hydrocarbons only receives a ready explanation if the participation of free ethyl radicals is admitted. During recent years some remarkable developments have been revealed by M. S. Kharasch and his co-workers in a series of papers on the factors which influence the course and mechanism of Grignard reactions. These investigations have shown that by means of the catalytic action of certain metallic halides the usefulness of Grignard reagents for preparative purposes can be very considerably extended and in addition a convenient method is provided for the generation of free radicals in solution.

[78] *J. Amer. Chem. Soc.*, 1943, **65**, 2446. [79] *Ibid.*, 1944, **66**, 1602.
[80] G. Williams, *J.*, 1940, 775.

Results so far obtained indicate that the Grignard reagent first reacts with the metallic halide (usually cobaltous chloride), thus : $RMgBr + CoCl_2 \longrightarrow RCoCl + MgBrCl$, and the intermediate RCoCl can then react in two ways : (a) $2RCoCl \longrightarrow R_2 + 2CoCl$, and (b) $RCoCl \longrightarrow R\cdot + CoCl$. The cobalt sub-halide can also react with organic halides to generate a free radical as follows : $R'Hal + CoCl \longrightarrow CoClHal + R'\cdot$.[81] The subsequent reactions of these free radicals in ethereal solution follow in general the known characteristics of free radicals in solution. It has also been shown that certain reactions of organo-lithium compounds are influenced by the presence of metallic halides.[82, 83]

M. S. Kharasch and E. K. Fields [81] observed that small quantities of certain metal halides have a marked effect on the reactions between aromatic Grignard reagents and organic halides. Neither arylmagnesium halides nor metal halides, such as $CoCl_2$, $NiCl_2$, $FeCl_3$, etc., react with aryl halides, but it is known that arylmagnesium halides will react with approximately molecular proportions of such metal halides to give biaryls. On the other hand, in the presence of relatively small quantities of the metal halide a vigorous reaction takes place between the arylmagnesium halide and the organic halide with the formation of a biaryl in good yield, *e.g.*, $PhMgBr + PhBr \xrightarrow{CoCl_2} Ph_2$. Both phenyl groups in the diphenyl molecule are derived from the Grignard reagent, since, when the bromobenzene is replaced by *p*-bromotoluene or by ethyl bromide, diphenyl is again formed in similar yield. The organic radical in the bromobenzene is responsible for the formation of traces of diphenyl, terphenyl, quaterphenyl, etc., since such polycyclic hydrocarbons are not formed when the bromobenzene is replaced by an aliphatic halide. Terphenyl, quaterphenyl, and higher polycylic hydrocarbons are always formed from free phenyl radicals in solution. This characteristic phenomenon, together with the fact that such small quantities of the metallic halide can effect biaryl formation, suggests a chain reaction, with the cobalt sub-halide as chain carrier, and the following mechanism was proposed :

$$\begin{aligned} PhMgBr + CoCl_2 &\longrightarrow PhCoCl + MgBrCl \\ 2PhCoCl &\longrightarrow Ph_2 + 2CoCl \\ CoCl + PhBr &\longrightarrow CoClBr + Ph\cdot \\ Ph\cdot &\longrightarrow PhH, Ph_2, Ph\cdot C_6H_4Ph, \text{ etc.} \end{aligned}$$

A study of the action of phenylmagnesium bromide on various aliphatic halides in presence and absence of cobaltous chloride was made by M. S. Kharasch, D. W. Lewis, and W. B. Reynolds,[84] which included an analysis of the gases formed from the free alkyl radicals liberated by the action of the cobalt sub-halide on the alkyl halide : .

$$CoCl + RBr \longrightarrow CoClBr + R\cdot$$

[81] M. S. Kharasch and E. K. Fields, *J. Amer. Chem. Soc.*, 1941, **63**, 2316.
[82] M. S. Kharasch and W. B. Reynolds, *ibid.*, p. 3239.
[83] M. S. Kharasch, D. W. Lewis, and W. B. Reynolds, *ibid.*, 1943, **65**, 498.
[84] *Ibid.*, p. 493.

In the absence of cobaltous chloride the reaction is very slow, no gases are evolved, no diphenyl is formed, and only the alkylbenzene can be isolated. On the other hand, in the presence of 3—5 molecules % of cobaltous chloride a vigorous reaction ensues. Very little alkylbenzene is formed, but diphenyl and hydrocarbon gases are produced in equivalent proportions, thus indicating a common origin, which can be represented thus :

$$\begin{aligned} PhMgBr + CoCl_2 &\longrightarrow PhCoCl + MgBrCl \\ 2PhCoCl &\longrightarrow Ph_2 + 2CoCl \\ CoCl + RBr &\longrightarrow CoClBr + R\cdot \end{aligned}$$

The free aliphatic radicals do not dimerise to any considerable extent but mainly undergo disproportionation to alkanes and alkenes. With methyl bromide the gases consist of 60% methane, 20% ethane, and 20% ethylene. The methane is derived from reaction between the methyl radical and the solvent (ether), whereas ethane and ethylene are attributed to the disproportionation of ethyl radicals also derived from the action of methyl radicals on the solvent, thus :

$$\begin{aligned} Me\cdot + EtOEt &\longrightarrow CH_4 + EtO\cdot\dot{C}H\cdot CH_3 \\ EtO\cdot\dot{C}H\cdot CH_3 &\longrightarrow Et\cdot + CH_3\cdot CHO \\ Me\cdot + EtOEt &\longrightarrow MeOEt + Et\cdot \\ 2Et\cdot &\longrightarrow C_2H_4 + C_2H_6 \end{aligned}$$

The influence of metallic halides on the action of Grignard reagents on a number of alicyclic chlorides has been studied by M. S. Kharasch, F. Engelmann, and W. H. Urry.[85] The reaction between methylmagnesium bromide and *cyclo*hexyl chloride is normally very slow and a secondary reaction (elimination of hydrogen chloride) predominates, but in the presence of 5 molecules % of cobaltous chloride a reaction takes place readily and the products consist of methane, ethane, and ethylene together with *cyclo*hexane, *cyclo*hexene, and di*cyclo*hexyl. The following mechanism is suggested :

$$\begin{aligned} &(a)\ MeMgBr + CoCl_2 \longrightarrow MeCoCl + MgBrCl \\ &(b)\ MeCoCl \longrightarrow Me\cdot + CoCl \\ &(c)\ RCl + CoCl \longrightarrow R\cdot + CoCl_2 \\ &(d)\ 2R\cdot \longrightarrow R_2 \\ &(e)\ 2R\cdot \longrightarrow R{-}H{+}R{+}H \\ &(f)\ Me\cdot + Et_2O \longrightarrow CH_4 \\ &(g)\ Me\cdot + Et_2O \longrightarrow Et\cdot + MeOEt \\ &(h)\ 2Et\cdot \longrightarrow C_2H_4 + C_2H_6 \end{aligned}$$

Although the dimerisation reaction (d) does not occur readily with the lower aliphatic radicals, it takes place to the extent of 26% with *cyclo*hexyl and to the extent of 63% with the bornyl radical.

A particularly interesting example of the dimerisation of free radicals is supplied by M. S. Kharasch and M. Kleiman,[86] who utilised the action of cobaltous chloride on a Grignard reagent to induce a free-radical chain

[85] *J. Amer. Chem. Soc.*, 1944, **66**, 365. [86] *Ibid.*, 1943, **65**, 491.

reaction with anethole hydrobromide, whereby dimerisation of the free radical results in the formation of the dimethyl ether of hexoestrol. The sequence of reactions may be represented as follows :

$$PhMgBr + CoCl_2 \longrightarrow PhCoCl + MgBrCl$$
$$PhCoCl \longrightarrow Ph\cdot + CoCl$$
$$p\text{-}MeO\cdot C_6H_4\cdot CHBrEt + CoCl \longrightarrow p\text{-}MeO\cdot C_6H_4\cdot \overset{|}{C}HEt + CoClBr$$
$$2p\text{-}MeO\cdot C_6H_4\cdot \overset{|}{C}HEt \longrightarrow p\text{-}MeO\cdot C_6H_4\cdot CHEt\cdot CHEt\cdot C_6H_4\cdot OMe\text{-}p$$

M. S. Kharasch, D. C. Sayles, and E. K. Fields [87] have made a further study of the action of aliphatic and aromatic Grignard reagents on certain mono- or di-halogeno-aromatic compounds in presence of cobaltous chloride. It is shown that in the examples studied the significant reaction is the replacement of halogen by hydrogen. Thus, butylmagnesium bromide reacts with bromobenzene in presence of 5 molecules % of cobaltous chloride to give benzene (44%), butane and butylene (83%), butylbenzene (3%), diphenyl (3%), and polyphenyls (11%). This reaction, which was applied to a wide variety of aromatic halogen compounds, is obviously one of wide applicability and the suggested mechanism involves a free-radical chain reaction, thus :

$$\text{(a) } RMgX + CoCl_2 \longrightarrow RCoCl + MgXCl$$
$$\text{(b) } RCoCl \longrightarrow R\cdot + CoCl$$
$$\text{(c) } ArBr + CoCl \longrightarrow Ar\cdot + CoClBr$$
$$\text{(d) } Ar\cdot + Et_2O \longrightarrow ArH$$

These results confirm the view that the dimerides of aromatic radicals are not formed in presence of a solvent by the simple combination of the two free aromatic radicals. The characteristic reaction of such radicals under these conditions is abstraction of hydrogen from the solvent, as previously established in the work of D. H. Hey and W. A. Waters.[2,3]

The influence of cobaltous chloride on the action of Grignard reagents on aromatic acyl halides has been studied by M. S. Kharasch, W. Nudenberg, and S. Archer.[88] Phenylmagnesium bromide and benzoyl chloride normally give benzophenone and triphenylcarbinol, but in the presence of 2 molecules % of cobaltous chloride the reaction is more complex and the products include ethyl benzoate, diphenyl, benzophenone, benzoic acid, phenylbenzoin, tetraphenylethylene oxide and stilbene dibenzoate. A mechanism involving free radicals with cobaltous sub-halide as chain carrier is proposed as follows :

$$PhMgBr + CoCl_2 \longrightarrow PhCoCl + MgBrCl$$
$$2PhCoCl \longrightarrow Ph_2 + CoCl$$
$$PhCOCl + CoCl \longrightarrow PhCO\cdot + CoCl_2$$

The various products formed in the reaction result from the interaction of the benzoyl radical with the various molecules in solution.

M. S. Kharasch, R. Morrison, and W. H. Urry [89] have investigated the

[87] *J. Amer. Chem. Soc.*, 1944, **66**, 481. [88] *Ibid.*, 1943, **65**, 495.
[89] *Ibid.*, 1944, **66**, 368.

reaction of methylmagnesium iodide with sterically hindered acid halides. When methylmagnesium iodide was added to 2 : 4 : 6-trimethylbenzoyl chloride, the product consisted of a mixture of mesityl methyl ketone (35%) and the substituted benzil (38%) :

$$2C_6H_2Me_3\cdot COCl + 2MeMgI \longrightarrow C_6H_2Me_3\cdot CO\cdot CO\cdot C_6H_2Me_3 + C_2H_6 \text{ (assumed)} + 2MgICl$$

If methylmagnesium bromide was used in place of the iodide, only traces of the benzil were formed even in the most favourable conditions. It thus appeared that the formation of the benzil was almost specific for the iodide. On the other hand, in presence of cobaltous chloride a fair yield of the benzil was obtained with methylmagnesium bromide. The fact that 2 : 4 : 6-trimethylbenzoyl chloride gave a substituted benzil with methylmagnesium iodide and with methylmagnesium bromide in presence of cobaltous chloride suggested that a common mechanism is involved. In the reaction with the cobaltous chloride the evidence indicates that the Grignard reagent is exercising its reducing action on the acid chloride by first forming the subhalide, which then functions as a chain carrier, thus :

$$\begin{aligned}
&\text{(a)}\ MeMgBr + CoCl_2 \longrightarrow MeCoCl + MgBrCl\\
&\text{(b)}\ MeCoCl \longrightarrow Me\cdot + CoCl\\
&\text{(c)}\ R\cdot COCl + CoCl \longrightarrow R\cdot CO\cdot + CoCl_2\\
&\text{(d)}\ 2R\cdot CO\cdot \longrightarrow R\cdot CO\cdot COR\\
&\text{(e)}\ Me\cdot + Et_2O \longrightarrow CH_4 + C_2H_6 + C_2H_4
\end{aligned}$$

If the reaction with methymagnesium iodide follows a similar course, no chain reaction is involved, but it is suggested that the iodide can react in two ways by means of either an unsymmetrical or a symmetrical fission, thus :

$$\begin{aligned}
&\text{(f)}\ MeMgI \longrightarrow Me^- + (MgI)^+\\
&\text{(g)}\ MeMgI \longrightarrow Me\cdot + MgI
\end{aligned}$$

In the above reactions with 2 : 4 : 6-trimethylbenzoyl chloride reaction (g) operates, thus giving magnesious iodide, which, like the cobaltous subhalide, can function as the reducing agent. Methylmagnesium iodide (and not the bromide) is thus able to function by means of either a free-radical mechanism or an ionic mechanism. The ionic process is usually the faster and the free-radical mechanism only comes into play where substances which can be reduced by magnesious iodide are present and where the normal Grignard reaction with the organic molecule is comparatively slow.

Previous work had shown that the free hydrocarbon radicals generated in solution by means of the catalytic action of metallic halides on Grignard reagents differed markedly in their properties. The lower alkyl radicals undergo disproportionation, whereas such radicals as benzyl and benzhydryl dimerise. Free radicals derived from groups of intermediate negativity, such as *cyclo*hexyl and bornyl, undergo both disproportionation and dimerisation. A study of the β-phenylethyl radical in ethereal solution showed that

ethylbenzene and styrene were formed in equivalent quantities (*i.e.*, disproportionation), together with some αδ-diphenylbutane (*i.e.*, dimerisation). With this knowledge W. H. Urry and M. S. Kharasch [90] turned their attention to the properties of the β-phenyl-ββ-dimethylethyl or neophyl radical in ethereal solution, the constitution of which does not permit disproportionation. Phenylmagnesium bromide and neophyl chloride did not react, but in presence of cobaltous chloride the products were *tert.*-butylbenzene, *iso*-butylbenzene, *iso*butenylbenzene, ββ-dimethylstyrene, dimerides $(C_{10}H_{13})_2$, and diphenyl. These results can only be explained by assuming the rearrangement of the neophyl free radical to a new structure which permits disproportionation to take place. The formation of these products is attributed to the following reactions :

(a) $PhMgBr + CoCl_2 \longrightarrow PhCoCl + MgBrCl$

(b) $2PhCoCl \longrightarrow Ph_2 + 2CoCl$

(c) $Ph{\cdot}CMe_2{\cdot}CH_2Cl + CoCl \longrightarrow Ph{\cdot}CMe_2{\cdot}CH_2{\cdot} + CoCl_2$

(d) $Ph{\cdot}CMe_2{\cdot}CH_2{\cdot} + Et_2O \longrightarrow Ph{\cdot}CMe_3$

(e) $Ph{\cdot}CMe_2{\cdot}CH_2{\cdot} \longrightarrow {\cdot}CMe_2{\cdot}CH_2Ph$ (i); $\longrightarrow Ph{\cdot}CMe{:}CH_2Me$ (ii)

(f) $2Ph{\cdot}CH_2{\cdot}CMe_2 \longrightarrow Ph{\cdot}CH_2{\cdot}CMe{:}CH_2 + Ph{\cdot}CH_2{\cdot}CHMe_2$; $\longrightarrow Ph{\cdot}CH{:}CMe_2 + Ph{\cdot}CH_2{\cdot}CHMe_2$

There is as yet no evidence of the rearrangement (e ii), but the rearrangement (e i) is regarded as the first unambiguous demonstration of the rearrangement in solution of a free radical involving the carbon skeleton.

D. H. H.

7. Chemistry of Nucleosides and Nucleotides.

Nucleotides related to Nucleic Acids.

In view of the rapidly increasing recognition [1] of the biological importance of nucleic acids and the related nucleotides and nucleosides it seems desirable at this stage to review the advances [2] made in the chemistry of these compounds since the publication of P. A. Levene and W. Bass' " Nucleic Acids," [3] which contains an authoritative account of work in this field before 1932. The chemistry of the nucleic acids themselves, which are highly polymerised compounds, may be considered under two headings : (1) chemistry of the nucleotide units from which the nucleic acid (polynucleotide) is built up ; (2) mode of union of the individual nucleotide units in the polynucleotide molecule. This review will be concerned only with work relating

[90] *J. Amer. Chem. Soc.*, 1944, **66**, 1438.

[1] For summary and references, see J. M. Gulland, Tilden Lecture, *J.*, 1944, 208.

[2] Recent reviews have been published by J. M. Gulland, *J.*, 1938, 1722 and ref. 1 ; H. Bredereck, " Fortschritte der Chemie Organischer Naturstoffe," L. Zechmeister, Wien, 1938, **1**, 121.

[3] American Chemical Society Monograph, New York, 1931.

to the first heading, discussion of the second problem (which is as yet not completely solved) being deferred to a future Report.

Controlled hydrolysis of ribonucleic acids (*e.g.*, yeast nucleic acid), which are of cytoplasmic origin,[4] furnishes four ribonucleotides, *viz.*, guanylic (I, R = PO_3H_2), yeast adenylic (II, R = PO_3H_2), cytidylic (III, R = PO_3H_2), and uridylic (IV, R = PO_3H_2) acids; by dephosphorylation of these, four ribonucleosides, guanosine, adenosine, cytidine and uridine (I—IV, R = H), respectively, are obtained. The ribonucleotides xanthylic acid and inosine phosphoric acid are obtained from (I and II, R = PO_3H_2), respectively, by deamination with nitrous acid, and the corresponding ribonucleosides xanthosine and inosine arise from similar treatment of (I and II, R = H).

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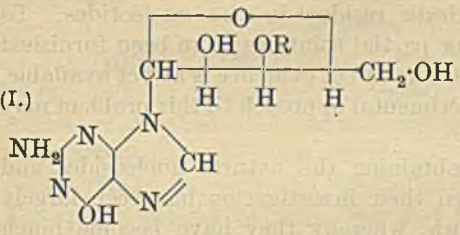

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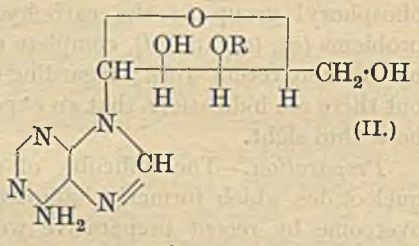

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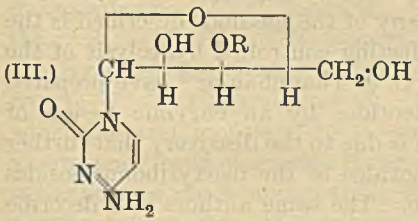

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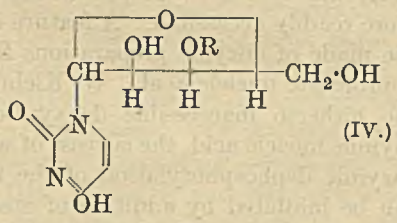

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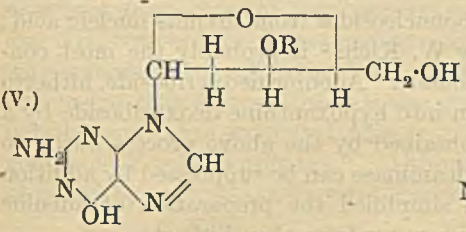

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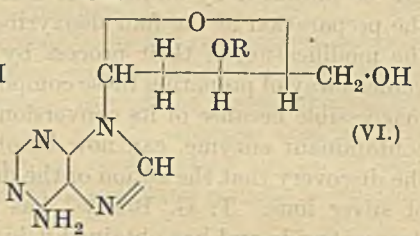

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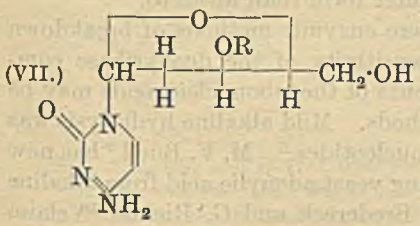

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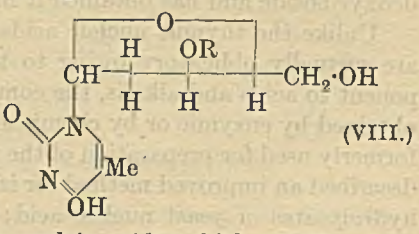


Deoxyribonucleic acids (*e.g.*, thymus nucleic acid), which are the characteristic acids of nucleal material,[4] give in a similar manner four deoxyribonucleotides, *viz.*, guanine-, adenine-, cytosine-, and thymine-(5-methyl-

[4] For references, see A. E. Mirsky, "Chromosomes and Nucleoproteins" in *Advances in Enzymology*, New York, 1943, **3**, 1.

uracil)-deoxyribosidephosphoric acids (V—VIII, R = PO_3H_2), from which by dephosphorylation the corresponding deoxyribonucleosides (V—VIII, R = H) are obtainable; hypoxanthinedeoxyriboside is known as the product of enzymic deamination of (VI, R = H). Earlier work had established the nature of the ribo- and deoxyribo-nucleosides as *N*-*d*-ribosides and *N*-*d*-2-deoxyribosides of the pyrimidine and purine bases after which they are named; the nucleotides were known to be the corresponding *N*-glycosidephosphoric esters. Four main structural problems therefore remained for investigation: (*a*) The point of attachment of the glycosidic radicals to the aglycons, (*b*) the configuration at the glycosidic linkage, (*c*) the size of the lactol ring, and (*d*) the position of attachment of the phosphoryl group to the carbohydrate residue in the nucleotides. To problems (*a*), (*c*), and (*d*), complete or partial solutions have been furnished by the more recent work; regarding (*b*) no direct evidence is as yet available, but there are indications that an experimental approach to this problem may be within sight.

Preparation.—The difficulty of obtaining the natural nucleosides and nucleotides which formerly hampered their investigation has been largely overcome by recent preparative work, whereby they have become much more readily accessible. A feature of many of the methods described is the use made of enzyme preparations for effecting controlled hydrolysis of the appropriate nucleic acid. W. Klein and S. J. Thannhauser [5] have prepared the hitherto inaccessible deoxyribonucleotides by an enzymic fission of thymus nucleic acid, the success of which is due to the discovery that further enzymic dephosphorylation of the nucleotides to the deoxyribonucleosides can be inhibited by addition of arsenate. The same authors also describe the preparation of the four deoxyribonucleosides from thymus nucleic acid; the modification of their process by W. Klein [6] is probably the most convenient way of preparing these compounds. Adenine deoxyriboside, hitherto inaccessible because of its conversion into hypoxanthine deoxyriboside by a contaminant enzyme, can now be obtained by the above process owing to the discovery that the action of the deaminase can be suppressed by addition of silver ions. T. G. Brady [7] has simplified the preparation of adenine deoxyriboside and has obtained it in a purer form than hitherto.

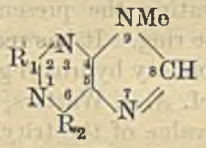
Unlike the thymus nucleic acids, where enzymic methods of breakdown are virtually obligatory owing to the sensitivity of the deoxyribose component to acids and alkalis, the components of the ribonucleic acids may be obtained by enzymic or by chemical methods. Mild alkaline hydrolysis was formerly used for preparation of the ribonucleotides.[8] M. V. Buell [9] has now described an improved method for isolating yeast adenylic acid from alkaline hydrolysates of yeast nucleic acid; H. Bredereck and G. Richter [10] claim that cytidylic and uridylic acids are most conveniently isolated by conducting the hydrolysis with dilute acid and separating these two acids by virtue of

[5] *Z. physiol. Chem.*, 1934, **224**, 244; 1935, **231**, 96, 125. [6] *Ibid.*, 1938, **255**, 82.
[7] *Biochem. J.*, 1941, **35**, 855. [8] P. A. Levene, *J. Biol. Chem.*, 1919, **40**, 415.
[9] *Ibid.*, 1943, **150**, 389. [10] *Ber.*, 1938, **71**, 718.

their differential solubility in pyridine–water. Nucleotides are also obtained [11] by hydrolysis of yeast nucleic acid with the crystalline ribonuclease of M. Kunitz,[12] but the hydrolysis is incomplete. On the other hand, a very convenient method of obtaining the four ribonucleosides by enzymic hydrolysis of yeast nucleic acid has been worked out in H. Bredereck's laboratory.[13, 14, 15] Of great interest also is the discovery [15] that hydrolysis of the nucleic acid can be carried out by boiling with aqueous pyridine; in many laboratories this procedure will be found more convenient than the enzymic method. The accessibility of adenosine is greatly increased by the description of an improved method for regenerating this nucleoside from its picrate [16]; the method may also be of value in the decomposition of other picrates.

Structure of Nucleosides.

(a) *Position of Attachment of the Glycosidic Radical to the Base.*—Before 1932, work on the purine nucleosides had established that the glycosidic radical is attached to one of the nitrogen atoms of the iminazole ring. The early assumption that this attachment involved N_7 has now been corrected by the important work of J. M. Gulland and collaborators. The ultra-violet absorption spectra of adenosine and inosine closely resemble those of 9-methyladenine (IX; $R_1 = H$, $R_2 = NH_2$) and 9-methylhypoxanthine (IX; $R_1 = H$, $R_2 = OH$) respectively, but differ markedly from those of the corresponding 7-methyl derivatives (X; $R_1 = H$, $R_2 = NH_2$ and OH resp.); J. M. Gulland and E. R. Holiday [17] therefore concluded that adenosine and inosine were 9- and not 7-glycosides. In the same way, xanthosine [18] and guanosine [19] show ultra-violet absorption spectra identical with those of the 9-methyl (IX; $R_2 = OH$, $R_1 = OH$ and NH_2 resp.) but divergent from those of the 7-methyl derivatives (X; $R_2 = OH$, $R_1 = OH$ and NH_2 resp.) of the corresponding aglycons. Similar results were obtained with adenine deoxyriboside [20] and guanine deoxyriboside,[19] leading to the conclusion that 9-substitution is the general rule in the purine nucleosides; parallel work has extended this result to muscle adenylic acid and the adenosine-5′-polyphosphates.[17] Although no confirmation has so far been obtained by chemical methods, these results have found general acceptance.



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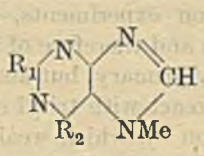

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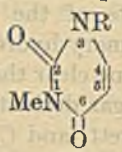


(XI.)

[11] H. S. Loring and F. H. Carpenter, *J. Biol. Chem.*, 1943, **150**, 381.
[12] *J. Gen. Physiol.*, 1940, **24**, 15.
[13] H. Bredereck, H. Beuchelt, and G. Richter, *Z. physiol. Chem.*, 1936, **244**, 102.
[14] H. Bredereck and G. Rothe, *Ber.*, 1938, **71**, 408.
[15] H. Bredereck, A. Martini, and F. Richter, *ibid.*, 1941, **74**, 694.
[16] H. Bredereck, *ibid.*, 1938, **71**, 1013. [17] *J.*, 1936, 765.
[18] J. M. Gulland, E. R. Holiday, and T. F. Macrae, *ibid.*, 1934, 1639.
[19] J. M. Gulland and L. F. Story, *ibid.*, 1938, 692. [20] *Idem*, *ibid.*, p. 259.

Determination of the position of attachment of the ribose residue in cytidine and uridine has been carried out by chemical methods. P. A. Levene and R. S. Tipson [21] converted uridine by an indirect method into an *N*-methyluridine (XI, R = $C_5H_9O_4$), which on complete hydrolysis gave 1-methyluracil (XI, R = H). Since the *N*-glycosidic nature of uridine was established by earlier work,[22] the ribose residue must be attached to N_3 in the uridine molecule. Uridine is obtained by deamination of cytidine, or from cytidylic acid by deamination to uridylic acid,[23] followed by dephosphorylation, so that a similar conclusion applies to each of these four compounds. H. Bredereck, G. Müller, and E. Berger [24] have sought to obtain evidence of the location of the sugar residue in the deoxyribonucleosides along similar lines. Fully methylated thymonucleic acid was subjected to acid hydrolysis, after which three different fission fragments could be isolated. The identity of one of these with 1-methylthymine demonstrates that in thymine deoxyriboside the glycosidic radical is attached to N_3 of the base. Other fragments isolated in the same way were a dimethylcytosine and a dimethyladenine; compounds identical with these were obtained by methylation and hydrolysis of cytidine and adenosine respectively. It does not appear to the writer valid to conclude on these grounds that the deoxyribose residue in cytosine- and adenine-deoxyribosides occupies the same position as does the ribose residue in the corresponding ribonucleosides (although this is probably true), since the 1 : N^6-dimethyl-cytosine and -adenine structures assigned to the acidic fission products by the authors were unsupported by any convincing evidence.

(b) *Size of the Lactol Ring.*—Rigid evidence of the furanoside nature of guanosine [25] and adenosine [26] was obtained by P. A. Levene and R. S. Tipson; complete methylation of these nucleosides, followed by acid hydrolysis, gave the same 2 : 3 : 5-trimethyl ribose, the structure of which was shown by its oxidation to a γ-lactone which on further oxidation yielded only *i*-dimethoxysuccinic acid. A modification of this method enabled the same authors to establish the furanoside structure of uridine; [27] the same conclusion is valid for cytidine.

The formation of trityl derivatives by the ribonucleosides confirms the results of the methylation experiments, demonstrating the presence of a primary hydroxyl group and therefore of a furanose ring. It has recently become clear that not only primary, but also the secondary hydroxyl groups of sugar derivatives may react with trityl chloride (cf. A. J. Watters, R. C. Hockett, and C. S. Hudson [28]), which weakens the value of the tritylation procedure as independent evidence of furanose structure in the nucleoside group, although H. Bredereck [29] found that (α and β) methylribopyranoside

[21] *J. Biol. Chem.*, 1934, **104**, 385.
[22] P. A. Levene and F. B. LaForge, *Ber.*, 1912, **45**, 619.
[23] H. Bredereck, *Z. physiol. Chem.*, 1934, **224**, 79.
[24] *Ber.*, 1940, **73**, 1058.
[25] *J. Biol. Chem.*, 1932, **97**, 491.
[26] *Ibid.*, 1932, **94**, 809.
[27] *Ibid.*, 1933, **101**, 529.
[28] *J. Amer. Chem. Soc.*, 1939, **61**, 1528.
[29] *Z. physiol. Chem.*, 1934, **223**, 61.

failed to react with trityl chloride under conditions successful for tritylation of the nucleosides. Monotrityl uridine [30] is probably a 5′-trityl derivative, since it can be converted [31] into a dimethyltosyluridine in which the tosyl group occupies the same position as did the original trityl group; the tosyl group in this compound can be exchanged for an iodine atom by the Oldham–Rutherford [32] method, a reaction commonly considered to be characteristic of primary tosyl esters. Monotrityladenosine [33, 34] is a 5′-trityl derivative; since it is stable to alkali, its formation cannot involve the amino-group of the adenine residue; furthermore, inosine, the deamination product of adenosine, also reacts with trityl chloride. Muscle adenylic acid, which is undoubtedly adenosine-5′-phosphate, has been obtained [34, 35] from trityladenosine through the stages 2′ : 3′-diacetyltrityladenosine, 2′ : 3′-diacetyladenosine and 2′ : 3′-diacetyladenosine-5′-phosphate, which shows the location of the original trityl group at $C_{5'}$, and this evidence finds support in the fact that N^6 : 2′ : 3′-tritosyltrityladenosine, obtained by tosylation of trityladenosine, gives with 80% acetic acid N^6 : 2′ : 3′-tritosyladenosine,[34] in which none of the tosyl groups will undergo exchange with iodine by the Oldham–Rutherford method. Tritylcytidine [33, 35] is also regarded as a 5′-trityl derivative; the trityl group is stable to alkali, so it is unlikely that it is an *N*-trityl derivative. Tritylguanosine cannot be prepared by direct reaction on account of the insolubility of guanosine in pyridine, but it has been obtained by an indirect method.[36]

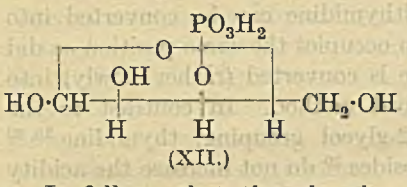
The trityl derivatives of the ribonucleosides have been considered in some detail above, since the successful tritylation of thymidine (thymine deoxyriboside) by P. A. Levene and R. S. Tipson [37] constitutes the most direct evidence available that this (or any other) deoxyribonucleoside possesses the furanose structure. Tritylthymidine can be converted into a tosylthymidine in which the tosyl group occupies the same position as did the original trityl group; tosylthymidine is converted (rather slowly) into iodothymidine by the Oldham–Rutherford method. In contrast to the ribonucleosides which possess a *cis*-1 : 2-glycol grouping, thymidine [38, 39] and guanine and hypoxanthine deoxyribosides [39] do not increase the acidity of boric acid in the Böeseken [40] reaction. Although on these grounds, and by analogy with the ribonucleosides, it is usually accepted that the deoxyribosides possess the furanose structure, a more rigid proof would appear desirable.

A method has recently been developed by B. Lythgoe and A. R. Todd [41]

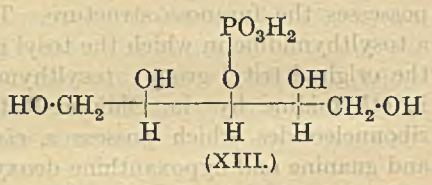
[30] H. Bredereck, *Ber.*, 1932, **65**, 1830; cf. ref. 21.
[31] P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, 1934, **105**, 419.
[32] J. W. H. Oldham and J. K. Rutherford, *J. Amer. Chem. Soc.*, 1932, **54**, 366.
[33] H. Bredereck, *Ber.*, 1933, **66**, 198.
[34] P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, 1937, **121**, 131.
[35] H. Bredereck, E. Berger, and J. Ehrenberg, *Ber.*, 1940, **73**, 269.
[36] H. Bredereck and E. Berger, *ibid.*, p. 1124. [37] *J. Biol. Chem.*, 1935, **109**, 623.
[38] P. A. Levene and R. S. Tipson, *Z. physiol. Chem.*, 1935, **234**, V.
[39] K. Makino, *Biochem. Z.*, 1935, **282**, 263.
[40] J. Böeseken, *Ber.*, 1913, **46**, 2612. [41] *J.*, 1944, 592.

for the determination of the lactol ring structure of nucleosides. Periodate oxidation of purine glycosides in which the sugar residue is attached to one of the nitrogen atoms of the iminazole ring was shown to follow a course identical with that demonstrated for the simpler *O*-glycosides by C. S. Hudson and collaborators [42]; application of the method permits a ready determination of the ring structure in such *N*-glycosides. It seems likely that this method will prove of considerable value in structural investigations in the nucleoside and nucleotide fields; by its aid it should be possible to verify the assumed furanoside structure of the deoxyribosides from thymus nucleic acid, to secure information regarding the location of the phosphoryl residues in adenosine polyphosphoric derivatives (*e.g.*, A T P and T P N) and to cast light on the problem of the configuration (α-, β-nature) of the glycosidic linkage in the pyrimidine and purine ribonucleosides.

(c) *Location of the Phosphoryl Residue in Nucleotides.*—Inosinephosphoric acid [43] and xanthylic acid [44, 45] (from deamination of yeast adenylic and guanylic acids respectively) undergo spontaneous hydrolysis in aqueous solution, giving the two bases, and one and the same *d*-ribosephosphoric acid (XII). For the location of the phosphoryl residue in this acid positions 2 and 3 are possible; positions 4 and 5 are excluded, since 4 is the point of closure of the lactol ring, and the *d*-ribose-5-phosphoric acid already known as the breakdown product of inosinic acid [46] is different from (XII). P. A. Levene and S. A. Harris [47] showed that (XII) gave on reduction a *d*-ribitol phosphoric acid (XIII), the optical inactivity of which demonstrates its symmetry; by contrast, *d*-ribitol-5-phosphoric acid, obtained from *d*-ribose-5-phosphoric acid by P. A. Levene, S. A. Harris, and E. T. Stiller,[48] is optically active.



(XII.)



(XIII.)

It follows that the phosphoryl residue esterifies the hydroxyl group at C_3' in the purine ribonucleotides. A similar location is probable in the pyrimidine ribonucleotides; P. A. Levene and E. Jorpes [49] found that dihydrocytidylic acid undergoes dephosphorylation at about the same speed as yeast adenylic and guanylic acids and H. Bredereck [50] showed that a

[42] E. L. Jackson and C. S. Hudson, *J. Amer. Chem. Soc.*, 1937, **59**, 994; 1939, **61**, 1530; W. D. Maclay and C. S. Hudson, *ibid.*, 1938, **60**, 2059; W. D. Maclay, R. M. Hann, and C. S. Hudson, *ibid.*, 1939, **61**, 1660; N. K. Richtmyer and C. S. Hudson, *ibid.*, 1943, **65**, 64.

[43] P. A. Levene and S. A. Harris, *J. Biol. Chem.*, 1933, **101**, 419.

[44] P. A. Levene and A. Dmochowski, *ibid.*, 1931, **93**, 563.

[45] P. A. Levene and S. A. Harris, *ibid.*, 1932, **95**, 755.

[46] P. A. Levene and W. A. Jacobs, *Ber.*, 1911, **44**, 746.

[47] *J. Biol. Chem.*, 1932, **98**, 9.

[48] *Ibid.*, 1934, **105**, 153.

[49] *Ibid.*, 1929, **81**, 579.

[50] *Z. physiol. Chem.*, 1934, **224**, 79.

trityluridylic acid could be obtained as the monobrucine or disodium salt and that cytidylic and uridylic acids gave no increase in acidity with boric acid in the Böeseken reaction. A uridine-5′-phosphate different from yeast uridylic acid has been obtained from uridine by a partial synthesis [36, 51] which demonstrates the 5′-location of the phosphoryl residue, and uridylic [36] and cytidylic [35] acids have both been prepared from the corresponding nucleosides by methods which limit the position of the phosphoryl group in these compounds to C_2' or C_3'; the latter alternative is usually accepted on the grounds of analogy with the purine nucleotides. A location of the phosphoryl group at C_3' is assumed for the deoxyribonucleotides; no evidence in confirmation of this assumption has yet been obtained.

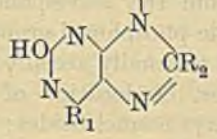
Other naturally occurring Nucleosides.—G. R. Barker and J. M. Gulland [52] have reinvestigated the sugar component present in the nucleosides of yeast nucleic acid by modern methods and confirm its identity with *d*-ribose. From a sample of commercial nucleic acid a small amount of *l*-lyxose was isolated as *l*-lyxobenziminazole, suggesting that nucleosides containing this sugar as component may occur naturally. H. S. Loring [53] isolated from hydrolysis of the pentosenucleic acid of tobacco mosaic virus the brucine salt of a uridylic acid isomeric and not identical with that from yeast nucleic acid; J. M. Gulland [1] has pointed out that some sugar component other than *d*-ribose may be present in the virus uridylic acid.

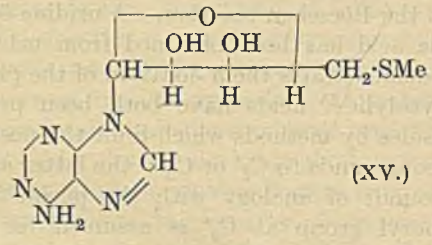
E. Cherbuliez and K. Bernhard [54] isolated from the cotton bean *Croton tiglium* a purine nucleoside crotonoside (XIV; $R_1 = NH_2$, $R_2 = H$) isomeric with guanosine and giving on hydrolysis *d*-ribose and *iso*guanine [54, 55, 56] (6-amino-2-hydroxypurine). R. Falconer, J. M. Gulland, and L. F. Story [57] deaminated crotonoside and showed by ultra-violet absorption methods that the ribose residue is attached to N_9 of the aglycon in both intact and deaminated crotonoside; the deaminated product is possibly identical with xanthosine. Uric acid riboside [58] (XIV; $R_1 = R_2 = OH$) has also been shown to be a 9-glycoside,[59] but the ring structure of the ribose residue has not yet been established either in this compound or in crotonoside. R. Falconer and J. M. Gulland [60] have shown that adenine thiomethyl pentoside [61] is a 9-glycoside, the corresponding hypoxanthine derivative has been prepared by R. Kuhn and K. Henkel,[62] and G. Wendt [63] has reinvestigated the sugar component; this gives 0·7 mol. of formaldehyde on treatment with lead tetra-acetate and the author considers that adenine thiomethyl

[51] P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, 1934, **106**, 113.
[52] *J.*, 1943, 625. [53] *J. Biol. Chem.*, 1939, **130**, 251.
[54] *Helv. Chim. Acta*, 1932, **15**, 464.
[55] J. R. Spies and N. L. Drake, *J. Amer. Chem. Soc.*, 1935, **57**, 774.
[56] J. R. Spies, *ibid.*, 1939, **61**, 350.
[57] *J.*, 1939, 1784.
[58] A. R. Davis, E. B. Newton, and S. R. Benedict, *J. Biol. Chem.*, 1922, **54**, 595.
[59] R. Falconer and J. M. Gulland, *J.*, 1939, 1369.
[60] *J.*, 1937, 1912.
[61] U. Suzuki, S. Odake, and T. Mori, *Biochem. Z.*, 1924, **154**, 278.
[62] *Z. physiol. Chem.*, 1941, **269**, 41. [63] *Ibid.*, 1942, **272**, 152.

pentoside has the structure (XV). F. Lipmann [64] has suggested that this nucleoside may be concerned with the biological transfer of methyl groups.

(XIV.) *d*-ribose





(XV.)

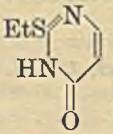
Synthesis of Nucleosides and Nucleotides.

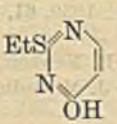
Increasing attention has lately been paid to the study of synthetic methods in this field. Successful outcome of such studies, as well as affording confirmation of structure, might, by rendering available compounds closely related to the natural members, further our knowledge of the relationship between chemical structure and biological action (*e.g.*, coenzyme action of the adenine derivatives). Partial synthesis of nucleotides by phosphorylation of the natural ribonucleosides has made progress; J. M. Gulland [1] has recently reviewed advances in this field. No naturally occurring nucleoside has yet been prepared synthetically, but close analogues have been obtained recently, both in the pyrimidine and in the purine series.

Two main types of method have been used in attempts to synthesise true nucleosides: (1) In the first type the complete pyrimidine or purine nucleus of the desired nucleoside is preformed in the starting material; by reaction of the latter or a silver derivative with an acetohalogen sugar a glycosidic radical is introduced into the nucleus, and the substituents present in the resulting pyrimidine or purine glycoside are then transformed into those characteristic of the natural nucleoside. (2) Alternatively a compound is chosen for glycosidisation which may afterwards be cyclised to a pyrimidine or purine derivative; this method has the advantage that the structure of the product may be demonstrated by the method of synthesis; it has so far been investigated only in the purine series.

Method 1.—One disadvantage of this type of synthesis is that owing to possibilities of tautomerisation in the nucleus the sugar residue may become attached preferentially to oxygen or non-nuclear nitrogen atoms; thus the glucoside of 2-ethylthiouracil (XVI *a* or *b*) [65] is shown by its lability to acids to be an *O*-glycoside and not a ring-*N*-glycoside. Other pyrimidine glycosides prepared by similar methods by E. Fischer [65] and by P. A. Levene and H. Sobotka [66] can be dismissed with the same remarks; they are not ring-*N*-glycosides.

(XVI*a*.)



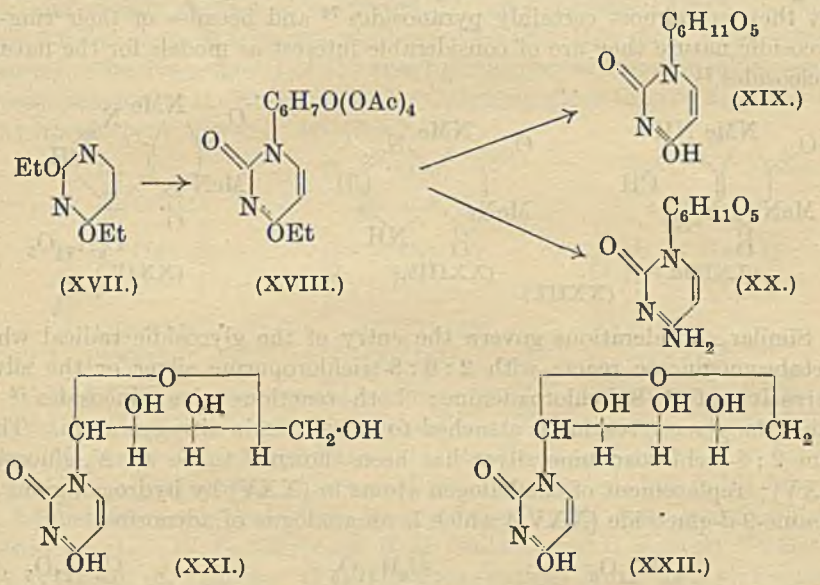


(XVI*b*.)

[64] *Advances in Enzymology*, New York, 1941, **1**, 100.

[65] E. Fischer, *Ber.*, 1914, **47**, 1377. [66] *J. Biol. Chem.*, 1925, **65**, 469.

This defect in the method can be overcome to some extent by choosing for glycosidisation pyrimidine derivatives in which possibilities of tautomerisation are restricted. G. E. Hilbert and T. B. Johnson [67] brought 2 : 6-diethoxypyrimidine (XVII) into reaction with acetobromoglucose to give the tetra-acetylglucoside (XVIII), from which the protecting ethyl and acetyl groups can be removed by alcoholic hydrogen chloride to give 3-glucosidouracil (XIX), an analogue of uridine (XXI), or by alcoholic ammonia to give 3-glucosidocytosine (XX),[68] an analogue of cytidine.

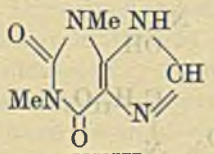


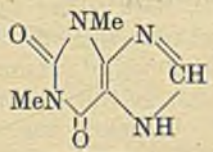
In the same way uracil-3-*d*-galactoside, -3-*d*-xyloside, -3-*l*-arabinoside [69] and -3-*d*-riboside [70] (XXII) were obtained. The last is of great interest, since it probably differs from uridine only in the size of the lactol ring; the above glycosides, like the acetohalogen sugars from which they were prepared, are almost certainly pyranosides. The structure of the products as N_3-glycosides rests mainly on the analogy of the reaction between 2 : 6-diethoxypyrimidine and methyl iodide, which gives 2-hydroxy-6-methoxy-3-methylpyrimidine,[71] but the synthetic glycosides resemble the natural nucleosides in their stability to acids; ready hydrolysis only becomes possible after hydrogenation to the 4 : 5-dihydro-derivatives.[22]

The limitation mentioned above is present when method (1) is applied to the synthesis of purine nucleosides. Theobromine-*d*-glucoside, prepared by E. Fischer and B. Helferich [72] from the reaction product of theobromine

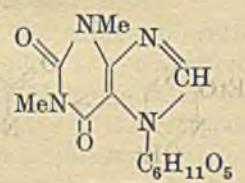
[67] *J. Amer. Chem. Soc.*, 1930, **52**, 4489.
[68] G. E. Hilbert and E. F. Jansen, *ibid.*, 1936, **58**, 60.
[69] G. E. Hilbert, *ibid.*, 1937, **59**, 330.
[70] G. E. Hilbert and C. E. Rist, *J. Biol. Chem.*, 1937, **117**, 371.
[71] G. E. Hilbert and T. B. Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 2001.
[72] *Ber.*, 1914, **47**, 210.

silver and acetobromoglucose, is an *O*-glycoside, but in theophylline (XXIII, *a* or *b*) the tautomeric possibilities restrict the position of entry of the sugar residue to one of the nitrogen atoms of the iminazole ring; the N_7-glycosidic nature of the theophylline glycosides (*e.g.*, XXIV) obtained by E. Fischer and B. Helferich's process has been established by J. M. Gulland and co-workers.[18] These glycosides are readily accessible: members have been prepared including hexosides,[72, 73] pentosides,[73, 65, 74] methyl pentosides,[75] a *d*-glucodesoside,[76] and a 5′-methyl rhamnofuranoside.[77] Apart from the last they are almost certainly pyranosides [74] and because of their ring-*N*-glycosidic nature they are of considerable interest as models for the natural nucleosides.[41]

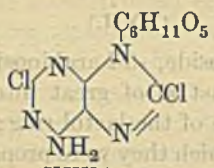

(XXIII*a*.)

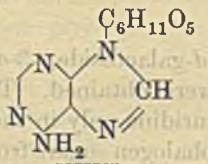

(XXIII*b*.)

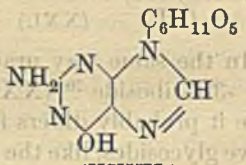
(XXIII.)


(XXIV.)

Similar considerations govern the entry of the glycosidic radical when acetobromoglucose reacts with 2 : 6 : 8-trichloropurine silver or the silver derivative of 2 : 8-dichloroadenine; both reactions give glucosides [72] in which the glucose residue is attached to an iminazole nitrogen atom. That from 2 : 8-dichloroadenine silver has been shown [20] to be an N_9-glucoside (XXV); replacement of the halogen atoms in (XXV) by hydrogen gives an adenine-9-*d*-glucoside (XXVI) which is an analogue of adenosine.


(XXV.)


(XXVI.)


(XXVII.)

Since (XXV) may also be converted into guanine glucoside (XXVII), J. M. Gulland and L. F. Story [20] proposed this method as a route to the synthesis of the naturally occurring nucleosides. Difficulties which might be encountered in a method of this type, apart from the disadvantage that the synthesis does not demonstrate the structure of the product, are (*a*) replacement of halogen atoms in (XXV) requires methods which might cause destruction of ribofuranose or deoxyribofuranose analogues and thus the

[73] B. Helferich and M. von Kühlewein, *Ber.*, 1920, **53**, 17.

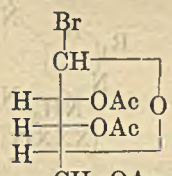
[74] J. Pryde and R. T. Williams, *J.*, 1933, 640.

[75] E. Fischer and K. von Fodor, *Ber.*, 1914, **47**, 1058; E. Fischer, O. Helferich, and P. Ostman, *ibid.*, 1920, **53**, 873; P. A. Levene and J. Compton, *J. Biol. Chem.*, 1937, **117**, 37.

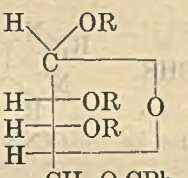
[76] P. A. Levene and F. Cortese, *ibid.*, 1931, **92**, 53.

[77] P. A. Levene and J. Compton, *ibid.*, 1936, **114**, 9.

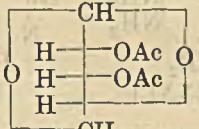
method might lack flexibility, (*b*) no preparation of an acetohalogenopentofuranose has yet been described in the literature, and in spite of the recently increased accessibility of *d*-ribose caused by publication of improved methods [14, 78, 79, 80] for its preparation, the problem of obtaining the acetohalogenoribofuranose (*e.g.*, XXVIII) necessary for application of this method (and that of G. E. Hilbert and coworkers [67–70]) to synthesis of the natural nucleosides is not an easy one. One unsuccessful attempt to obtain (XXVIII) for purposes of nucleoside synthesis is recorded by H. Bredereck and co-workers,[81] who converted 5-trityl α-*d*-ribose (XXIX, R = H) into 5-trityl 1 : 2 : 3-triacetyl *d*-ribose (XXIX, R = Ac), from which, however, selective removal of the trityl group proved impracticable, simultaneous scission of the acetyl group at C_1 taking place to give 2 : 3-diacetyl anhydroribose<1 : 5><1 : 4> (XXX).



(XXVIII.)



(XXIX.)



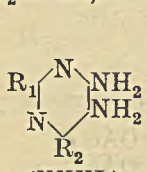
(XXX.)

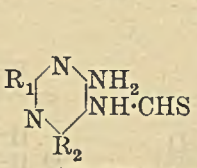
Method 2.—Two unsuccessful attempts by J. M. Gulland and co-workers to synthesise purine nucleosides belong to this category. In the first,[82] a possible synthesis of theophylline-9-*d*-glucoside along the lines of E. Fischer and L. Ach's [83] synthesis of uric acid was investigated, but failed because 1 : 3-dimethyluramil could not be condensed with tetra-acetyl glucose *iso*thiocyanate. W. E. Allsebrook, J. M. Gulland, and L. F. Story [84] describe the synthesis of xanthine (2 : 6-dihydroxypurine) from methyl 5-aminoglyoxaline-4-carboxylate by a method recalling J. Sarasin and E. Wegmann's earlier synthesis [85] of 7-methylxanthine; this synthesis of xanthine was intended as a model for the synthesis of xanthine-9-*d*-glucoside, but the *N*-glucosido-glyoxaline derivative required as starting material for the latter could not be obtained.

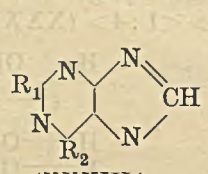
More successful have been model experiments on the synthesis of adenine derivatives, which have now led to the synthesis of 9-*d*-xylopyranosido-2-methyladenine, 9-*d*-xylopyranosidoadenine, and 9-*d*-ribopyranosidoadenine by a method demonstrating the constitution of the synthetic products. J. Baddiley, B. Lythgoe, D. McNeill, and A. R. Todd [86] considered that W. Traube's [87] well-known synthesis of adenine promised to possess the

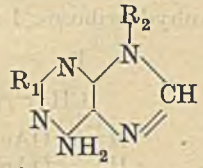
[78] F. P. Phelps, U.S. Pat. 2,152,662.
[79] M. Steiger, *Helv. Chim. Acta*, 1936, **19**, 189.
[80] R. Pasternack and E. V. Brown, U.S. Pat. 2,237,263.
[81] H. Bredereck, M. Kothnig, and E. Berger, *Ber.*, 1940, **73**, 956.
[82] J. M. Gulland and T. F. Macrae, *J.*, 1933, 662.
[83] *Ber.*, 1895, **28**, 2473; H. Biltz and K. Strufe, *Annalen*, 1921, **423**, 200.
[84] *J.*, 1942, 232.
[85] *Helv. Chim. Acta*, 1924, **7**, 713.
[86] *J.*, 1943, 383.
[87] *Annalen*, 1904, **331**, 64.

flexibility desirable in a method for the synthesis of purine nucleosides and model experiments were undertaken so that the steps in this synthesis might be carried out under conditions sufficiently mild to avoid the hydrolysis of glycosidic linkages when applied to analogues containing a sugar residue. It was found necessary to introduce three principal modifications. A suitable method for cyclisation of 4 : 5-diaminopyrimidine derivatives (XXXI) to the purine derivatives (XXXIII) was found [86] in that the thioformyl derivatives (XXXII) lost hydrogen sulphide on heating in anhydrous pyridine; this method was shown to be applicable to production of adenine itself (XXXIV; $R_1 = R_2 = H$),[88] 2-substituted adenine derivatives (XXXIV, $R_2 = H$), and 9-alkyladenine derivatives (*e.g.*, XXXIV; $R_1 = SMe$, $R_2 = Me$).[86]

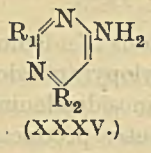

(XXXI.)

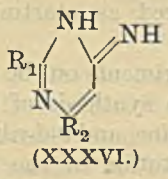

(XXXII.)

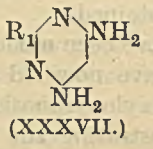

(XXXIII.)

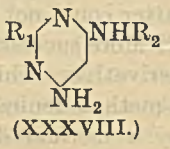

(XXXIV.)

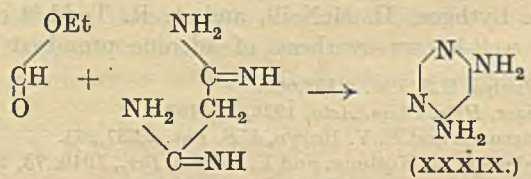
Attention was then directed [89] to the preparation of 4-glycosidaminopyrimidine derivatives. The tendency of 4-aminopyrimidine derivatives (XXXV) to behave as the tautomeric 4-iminodihydropyrimidine derivatives (XXXVI) imposes limitations on the possibility of glycosidising such compounds, but it was found that the 4 : 6-diaminopyrimidine derivatives (XXXVII), where R is some radical other than HO, SH, NH_2, etc., which are capable of prototropic change, could be converted into the 6-amino-4-glycosidaminopyrimidine derivatives (XXXVIII; $R_2 = C_5H_9O_4$ or $C_6H_{11}O_5$) by direct reaction with aldoses in presence of hydrogen chloride or ammonium chloride, a method employed by R. Kuhn and R. Ströbele [90] for the production of *N*-glycosides of the *o*-nitroaniline series.


(XXXV.)


(XXXVI.)

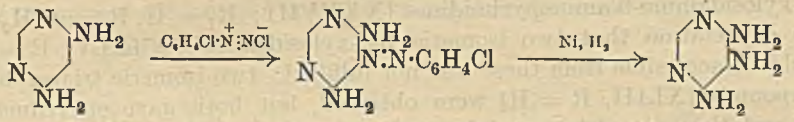

(XXXVII.)


(XXXVIII.)


(XXXIX.)

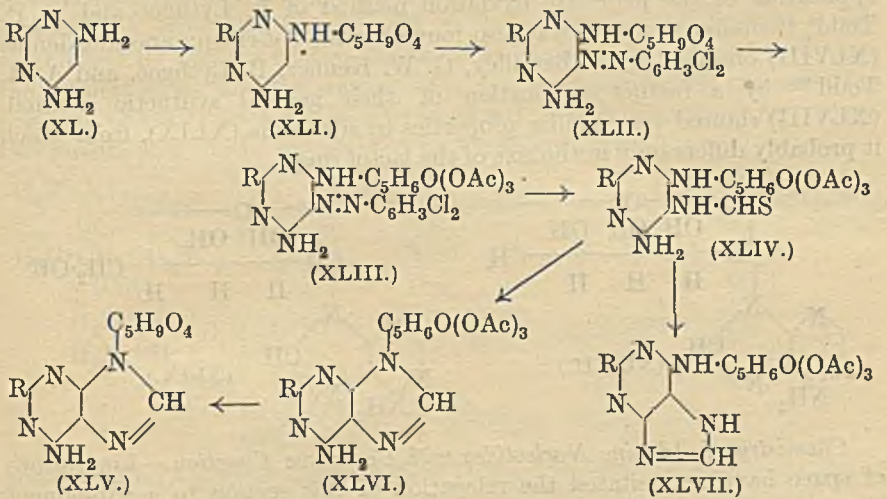
[88] J. Baddiley, B. Lythgoe, and A. R. Todd, *J.*, 1943, 386.
[89] *Idem*, *ibid.*, p. 571.
[90] *Ber.*, 1937, **70**, 773; R. Kuhn and L. Birkofer, *Ber.*, 1938, **71**, 621.

4 : 6-Diaminopyrimidine (XXXIX) itself, required as starting material for the synthesis of adenine-9-glycosides, was obtained [91] by condensing ethyl formate and malondiamidine, a method which represents a novel formation of the pyrimidine ring system,[92] but nitrosation of (XXXIX) and of (XXXVII, $R_1 = Me$) and the corresponding glycosides was found impossible except in presence of mineral acids, which caused hydrolysis of the latter. B. Lythgoe, A. R. Todd, and A. Topham [93] therefore investigated the reactions of a variety of sugar-free pyrimidine derivatives with reactive diazonium salts; they found that under suitable conditions coupling takes place at C_5 in the pyrimidine nucleus, and reductive fission of the azopyrimidines so obtained provides a convenient method for the preparation of 5-aminopyrimidine derivatives; *e.g.*,



In applying this method to the glycosides (XXXVIII; $R_1 = H$ or Me; $R_2 = C_5H_9O_4$) it was found desirable to protect the sugar hydroxyls by acetylating the 5-arylazopyrimidine (XLII) before reduction in order to prevent decomposition at a later stage in the synthesis.

J. Baddiley, B. Lythgoe, and A. R. Todd [94] employed the results obtained in the above investigations to effect a synthesis of 9-*d*-xylopyranosido-2-methyladenine (XLV, R = Me) by the method indicated schematically below :



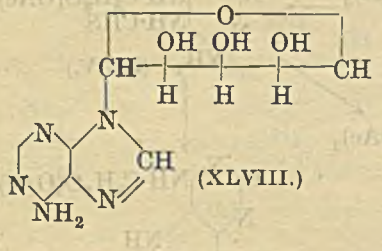
An unexpected feature of this synthesis is the simultaneous formation of 6-triacetyl-*d*-xylosidamino-2-methylpurine (XLVII, R = Me) and the

[91] G. W. Kenner, B. Lythgoe, A. R. Todd, and A. Topham, *J.*, 1943, 574.
[92] G. A. Howard, B. Lythgoe, and A. R. Todd, *J.*, 1944, 476.
[93] *Ibid.*, p. 315.
[94] *Ibid.*, p. 318.

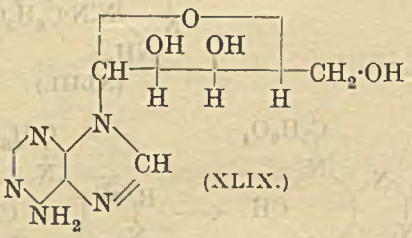
isomeric 9-*d*-xyloside (XLVI, R = Me); earlier work [86] had shown that cyclisation of the thioformyl derivative (XXXII; R_1 = SMe, R_2 = NHMe) gave rise to (XXXIV; R_1 = SMe, R_2 = Me); no evidence for formation of the theoretically possible alternative (XXXIII; R_1 = SMe, R_2 = NHMe) could be obtained. 6-*d*-Glycosidaminopurines such as that obtained from deacetylation of (XLVII, R = Me) contrast in behaviour with the isomeric 9-*d*-glycosidoadenine derivatives (XLV) in their greater lability to acids, their ready solubility in dilute alkali, and their failure to undergo deamination with nitrous acid.

G. W. Kenner, B. Lythgoe, and A. R. Todd [95] applied the method described above to synthesise 9-*d*-xylopyranosidoadenine (XLV, R = H); it was found that condensation of (XXXIX) with *d*-xylose gave two isomeric 4-*d*-xylosidamino-6-aminopyrimidines (XXXVIII; R_1 = H, R_2 = $C_5H_9O_4$). The expectation that two isomeric 9-*d*-xylosidoadenines (XLV, R = H) would be accessible from these was not fulfilled; two isomeric triacetylazo-compounds (XLIII, R = H) were obtained, but both gave on reduction one and the same 4-triacetyl-*d*-xylosidamino-5 : 6-diaminopyrimidine; the authors point out the remarkable nature of these changes, which they regard as due to an intraconversion of α, β-isomers. The location of the ultra-violet absorption maxima of (XLV, R = H and Me) lay closer to those of the 9-methyl- than to those of the 7-methyl-derivatives of the corresponding aglycons, thus supporting the conclusions drawn by J. M. Gulland and his collaborators.[17, 18, 19, 20]

The xylosides (XLV, R = H and Me) were shown to be pyranosides by application of the periodate oxidation method of B. Lythgoe and A. R. Todd; [41] similar structure was also found for the 9-*d*-ribopyranosidoadenine (XLVIII) obtained by J. Baddiley, G. W. Kenner, B. Lythgoe, and A. R. Todd [96] by a further application of their general synthetic method. (XLVIII) showed very similar properties to adenosine (XLIX), from which it probably differs only in the size of the lactol ring.



(XLVIII.)



(XLIX.)

Chemistry of Adenine Nucleotides with Coenzyme Function.—Limitations of space have necessitated the relegation of this section to a subsequent Report.

B. L.

[95] *J.*, 1944, 652.

[96] *Ibid.*, p. 657.

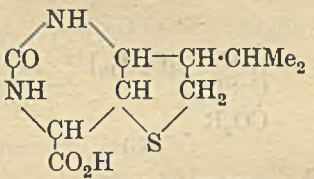
8. Heterocyclic Compounds.

The Biotins.

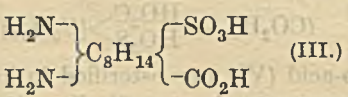
The discovery by F. Kögl and E. J. ten Ham [1] that the biotins occurring in egg-yolk and in liver are not identical was briefly reported last year.[2] F. Kögl has designated these substances α- and β-biotin respectively. The experimental evidence leading to the assignment of the structure (I) to α-biotin can now be reviewed.

Degradation of α-Biotin.—Earlier work [3] proved the presence of a cyclic urea group and a cyclic thio-ether linkage in α-biotin; moreover, oxidation of α-biotin methyl ester, and vigorous hydrolysis of the resulting sulphone (II), afforded a C_9-diaminosulphocarboxylic acid (III), thus proving that the urea group and the sulphur atom are situated in different rings.

(I.)

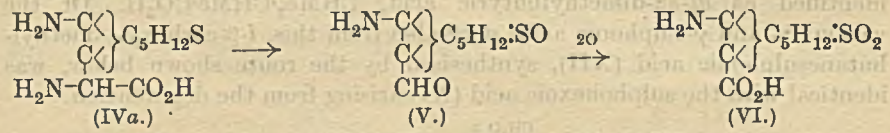


(II.)

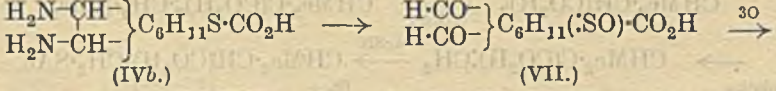


(III.)

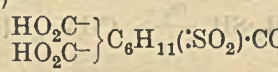
In order to obtain information concerning the position of the carboxyl group, F. Kögl, H. Erxleben, and J. H. Verbeek [4] studied the action of lead tetra-acetate on the diamino-carboxylic acid (IV) obtained by hydrolysis of α-biotin. A little more than 2 mols. of the reagent was consumed; it was established by model experiments that one molecule is required to oxidise the sulphur atom, and the consumption of a second molecule indicated the presence either of an α-amino-acid or of a 1 : 2-diamine structure. These two possibilities can be represented by the following schemes :



(IV*a*.) (V.) (VI.)



(IV*b*.) (VII.)



(VIII.)

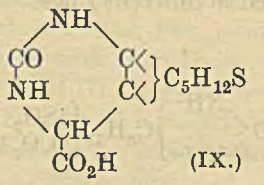
and a decision was made between them by carrying out a further oxidation of the aldehydic product with permanganate. In several experiments, 1·8 atomic proportions of oxygen were consumed, in approximate agreement with the transformation of (V) into (VI); cold esterification of the resulting

[1] *Z. physiol. Chem.*, 1943, **279**, 121. [2] *Ann. Reports*, 1943, **40**, 176.

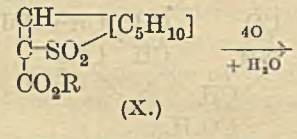
[3] F. Kögl and T. J. de Man, *Z. physiol. Chem.*, 1941, **269**, 82; *Ann. Reports*, 1943, **40**, 173. [4] *Z. physiol. Chem.*, 1942, **276**, 63.

acid afforded a small amount of a basic ester, supporting the structure (VI). Finally, the intermediate aldehyde (V) was isolated as a 2 : 4-dinitrophenylhydrazone of the correct nitrogen content.

From these results it appears certain that the lead tetra-acetate fission occurred between a carboxyl group and a carbon atom carrying an amino-group; the possibility that the substance (IV) is an αβ-diamino-carboxylic acid, and that fission occurred preferentially between the carboxyl group and the α-carbon atom, is almost certainly excluded by the observation of R. Criegee,[5] that even *trans*-1 : 2-glycols are attacked much more rapidly than α-hydroxy-acids. The urea group of α-biotin must therefore be contained in a six-membered ring, and the substance must be a derivative of 2-ketohexahydropyrimidine-4-carboxylic acid (IX).



(IX.)



(X.)

$$(CO_2H)_2 + \begin{matrix} HO_2C \\ HO_3S \end{matrix}\!\!>[C_5H_{10}] \quad \text{(XI.)}$$

When the amino-acid (VI) was esterified in hot solution, ammonia was eliminated and an unsaturated ester (X, R = Me) was obtained, in agreement with the behaviour of known β-amino-acids.[6] On oxidation of the derived acid (X, R = H) with permanganate, 4 atomic proportions of oxygen were consumed, and the product was a sulphocarboxylic acid (XI), isolated as its *m*-toluidine salt.[7] In order to identify the acid (XI), it was further degraded by alkali fusion,[8] model experiments having shown that β-sulphocarboxylic acids are converted into unsaturated carboxylic acids by this treatment. Hydrogenation of the product afforded a saturated acid, identified as *dl*-αβ-dimethylbutyric acid, $CHMe_2{\cdot}CHMe{\cdot}CO_2H$. Of the various β- and γ-sulphonic acids derivable from this, *l*-β-carboxy-γ-methylbutanesulphonic acid (XII), synthesised by the route shown below, was identical with the sulphohexoic acid (XI) arising from the degradation.

$$CHMe_2{\cdot}CH(CO_2H)_2 \xrightarrow[NHMe_2]{CH_2O+} CHMe_2{\cdot}C(CO_2H)_2{\cdot}CH_2{\cdot}NMe_2$$

$$\longrightarrow CHMe_2{\cdot}C(CO_2H){:}CH_2 \xrightarrow{AcSH} CHMe_2{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}SAc$$

$$\xrightarrow[\text{then aq. NaOH}]{\text{Resolution}} CHMe_2{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}SH \xrightarrow[H_2O]{Br_2+} CHMe_2{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}SO_3H \quad \text{(XII.)}$$

The above evidence thus leads to the structure (I) for α-biotin. The position assigned to the sulphur atom is considered to be supported by the great resistance to fission of the thio-ether linkage in the diamino-acid (IV),

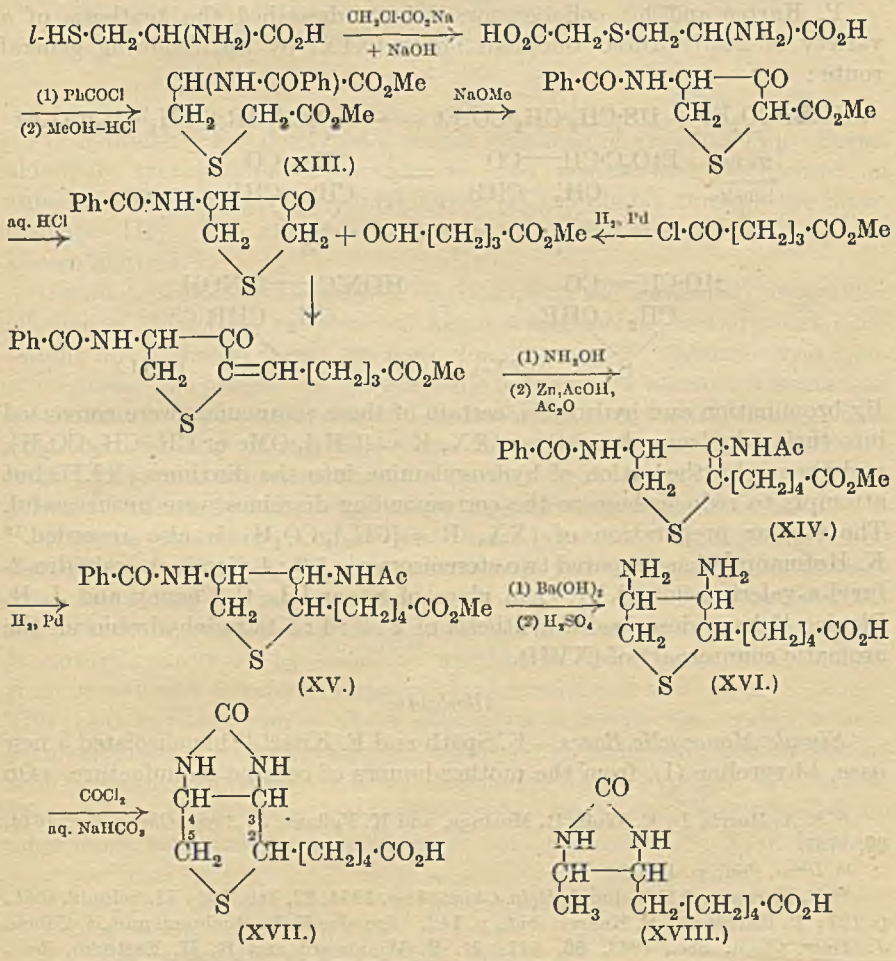
[5] *Ber.*, 1940, **73**, 563, 571. [6] C. Mannich and E. Ganz, *Ber.*, 1922, **55**, 3489.

[7] S. Zuffanti, *J. Amer. Chem. Soc.*, 1940, **62**, 1044.

[8] F. Kögl, J. H. Verbeek, H. Erxleben, and W. A. J. Borg, *Z. physiol. Chem.*, 1943, **279**, 121.

and by the relatively facile hydrolysis of the corresponding sulphone (II), both of which are attributed to the influence of the neighbouring carboxyl and amino-groups. The particularly ready fission of ammonia during the esterification of the β-amino-acid (VI) may also be due to the proximity of the sulphone group to the amino-group. The failure of the diamino-acid sulphone derived from β-biotin [9] to undergo hydrolytic fission may be attributable to the difference in the position of the carboxyl group in this substance.

Synthesis of β-*Biotin*.—Final confirmation of the structure (XVII) assigned to β-biotin [10] has been obtained by synthesis,[11] the method employed being outlined in the following scheme : [12]



[9] V. du Vigneaud *et al.*, *J. Biol. Chem.*, 1942, **145**, 101.
[10] *Ann. Reports*, 1943, **40**, 172.
[11] S. A. Harris, D. E. Wolf, R. Mozingo, and K. Folkers, *Science*, 1943, **97**, 447.
[12] *Idem*, *J. Amer. Chem. Soc.*, 1944, **66**, 1756.

Cyclisation of the di-ester (XIII) resulted in racemisation.[13] Hydrogenation of (XIV) afforded two racemic forms of (XV), from which were obtained *dl*-β-biotin and the stereoisomeric *dl-allo*biotin respectively. *dl*-β-Biotin was resolved through its ester with *l*-mandelic acid. The substance (XIV) was accompanied by a lower-melting isomer, which was converted by the same series of reactions into a mixture of *dl-allo*biotin and a third stereoisomer, *dl-epiallo*biotin. Both these isomers on hydrogenolysis with Raney nickel afford the same dethio*allo*biotin (XVIII), which is not identical with *dl*-dethiobiotin; hence they differ only in the configuration at C_2, whereas *dl*-biotin possesses the alternative relative configuration at C_3 and C_4.[14]

P. Karrer and his collaborators [15] have described the synthesis of a variety of 2-substituted thiophan-3-ones (XIX) by the following general route :

$$CHRBr{\cdot}CO_2Et + HS{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2Et \longrightarrow EtO_2C{\cdot}CHR{\cdot}S{\cdot}CH_2{\cdot}CH_2{\cdot}CO_2Et$$

$$EtO_2C{\cdot}CH(CH_2)CO \xrightarrow[\text{in PhMe}]{\text{NaOMe}} CH_2(CH_2)CO \quad \text{(XIX.)}$$
$$\qquad\qquad CHR \qquad\qquad CHR$$
$$\qquad\qquad S \qquad\qquad S$$

$$HO{\cdot}CH(CH_2)CO \qquad HON{:}C(CH_2)C{:}NOH$$
$$\qquad CHR \qquad\qquad CHR$$
$$\qquad S \quad \text{(XX.)} \qquad S \quad \text{(XXI.)}$$

By bromination and hydrolysis, certain of these compounds were converted into their 4-hydroxy-derivatives (XX, R = $[CH_2]_4{\cdot}OMe$ or $CH_2{\cdot}CH_2{\cdot}CO_2H$), and thence by the action of hydroxylamine into the dioximes (XXI), but attempts to reduce these to the corresponding diamines were unsuccessful. The similar preparation of (XX, R = $[CH_2]_4{\cdot}CO_2H$) is also recorded.[16] K. Hofmann [17] has prepared two stereoisomeric δ-3 : 4-diaminotetrahydro-2-furyl-*n*-valeric acids (XVI, O in place of S), and L. C. Cheney and J. R. Piening [18] have described a synthesis of 2 : 3 : 4 : 5-tetradehydrobiotin, the aromatic counterpart of (XVII).

Alkaloids.

Simple Monocyclic Bases.—E. Späth and F. Kittel [19] have isolated a new base, *l*-hygroline (I), from the mother-liquors of cocaine manufacture. On

[13] S. A. Harris, D. E. Wolf, R. Mozingo, and K. Folkers, *J. Amer. Chem. Soc.*, 1944, **66**, 1757.

[14] *Idem, ibid.*, p. 1800.

[15] P. Karrer and H. Schmid, *Helv. Chim. Acta*, 1944, **27**, 116, 124; H. Schmid, *ibid.*, p. 127; P. Karrer and F. Kehrer, *ibid.*, p. 142. See also E. R. Buchman and H. Cohen, *J. Amer. Chem. Soc.*, 1944, **66**, 847; R. B. Woodward and R. H. Eastman, *ibid.*, p. 849.

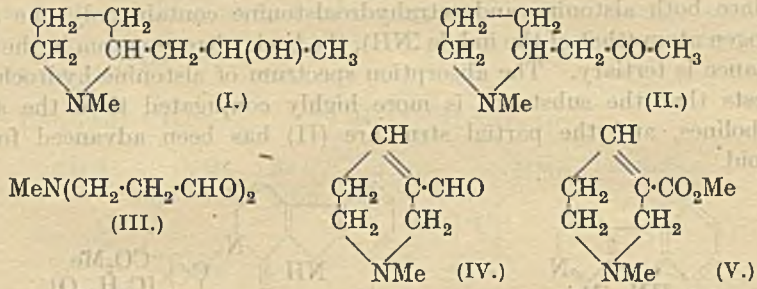
[16] P. Karrer, A. Keller, and E. Usteri, *Helv. Chim. Acta*, 1944, **27**, 237.

[17] *J. Amer. Chem. Soc.*, 1944, **66**, 157.

[18] *Ibid.*, p. 1040.

[19] *Ber.*, 1943, **76**, 942.

oxidation it afforded hygrine (II), which is now shown to be a racemate. The slight optical activity of (II) observed by C. Liebermann [20] is ascribed to the presence of an impurity (possibly I), since the oxime of Liebermann's material is inactive. The synthesis of *dl*-hygrine [21] thus constitutes a complete synthesis of the natural alkaloid.



C. Mannich [22] has described a simple synthesis of arecoline (V). Formaldehyde, methylamine hydrochloride, and acetaldehyde condensed in aqueous solution to give arecaidaldehyde (IV), probably through the intermediate (III). The aldehyde (IV) was then converted into (V) by the known method.[23]

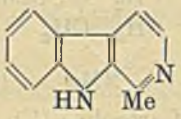
Indole Alkaloids.—Gelsemine, $C_{20}H_{22}O_2N_2$, the principal, crystalline alkaloid of *Gelsemium sempervirens* Ait, is a monoacidic, tertiary base, containing one hydroxyl [24] and one methylimino-group.[25] Catalytic hydrogenation reveals the presence of one ethylenic linkage; the alkaloid is isomerised by the action of platinised zinc and hydrochloric acid, the *iso*gelsemine thus obtained affording on hydrogenation the same dihydrogelsemine, which readily yields a dinitro-derivative.[26] On heating with soda-lime or selenium, gelsemine affords 2 : 3-dimethylindole, together with unidentified bases.[25]

Alstonia constricta contains the bases alstonine, $C_{21}H_{20}O_3N_2$,[27] and alstoniline, $C_{22}H_{18}O_3N_2$.[28] The former is a yellow, monoacidic, tertiary base, containing one methoxyl and no *N*-alkyl group. The free base, but not its salts, is reduced catalytically to colourless tetrahydroalstonine, which is slowly hydrolysed by alkali to amphoteric tetrahydroalstoninic acid, reconverted into tetrahydroalstonine by esterification with methyl alcohol. The third oxygen atom in these substances is inert. On oxidation with permanganate, the alkaloid affords *N*-oxalylanthranilic acid, and on heating with selenium an oxygen-free base, alstyrine, probably $C_{19}H_{22}N_2$, is obtained. Degradation of alstyrine methochloride by Emde's method afforded an amorphous base which exhibited indole colour reactions.[29] N. J. Leonard

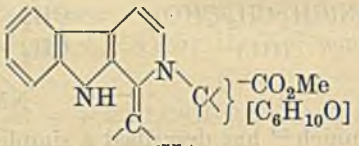
[20] *Ber.*, 1889, **22**, 675.
[21] K. Hess, *ibid.*, 1913, **46**, 3113, 4104.
[22] *Ibid.*, 1942, **75**, 1480.
[23] A. Wohl and A. Johnson, *ibid.*, 1907, **40**, 4712.
[24] C. W. Moore, *J.*, 1910, **97**, 2223.
[25] L. Marion, *Canadian J. Res.*, 1943, **21**, *B*, 247.
[26] T. T. Chu and T. Q. Chou, *J. Amer. Chem. Soc.*, 1940, **62**, 1955; 1941, **63**, 827.
[27] T. M. Sharp, *J.*, 1934, 287; cf. O. Hesse, *Ber.*, 1878, **11**, 1546.
[28] W. L. Hawkins and R. C. Elderfield, *J. Org. Chem.*, 1942, **7**, 573.
[29] T. M. Sharp, *J.*, 1938, 1353.

and R. C. Elderfield [30] observed that tetrahydroalstonine resembles yohimbine and other tetrahydro-β-carbolines in its behaviour in the modified Adamkiewicz test,[31] and also in its absorption spectrum, and confirmed the presence of the β-carboline nucleus by the isolation of harman (I), norharman and indole-2-carboxylic acid from the products of potash fusion.

Since both alstonine and tetrahydroalstonine contain only one active hydrogen atom (that of the indole :NH), the basic nitrogen atom in the latter substance is tertiary. The absorption spectrum of alstonine hydrochloride suggests that the substance is more highly conjugated than the simple β-carbolines, and the partial structure (II) has been advanced for the alkaloid.



(I.)



(II.)

Alstoniline is extremely sensitive to atmospheric oxidation, whereby it is converted into a crystalline " oxide," $C_{22}H_{18}O_4N_2,H_2O$. On catalytic hydrogenation the alkaloid absorbed two molecular proportions of hydrogen, but the product could not be isolated, as it was very rapidly re-oxidised by the air. The salts are more stable and give tetrahydro-derivatives which are stable, and thus appear to possess a different structure.[28]

Rauwolscine, $C_{21}H_{26}O_3N_2$, isolated from the leaves of *Rauwolfia canascens*, also appears to be related in structure to yohimbine, with which it is isomeric, and which it resembles in its colour reactions and absorption spectrum. Rauwolscine is a methyl ester, converted by hydrolysis into rauwolscinic acid, which yields harman (I) and 3-ethylindole on pyrolysis; alkali fusion produces harman, indole-2-carboxylic acid, and *iso*phthalic acid. The alkaloid forms a monoacetyl derivative, and contains two active hydrogen atoms.[32]

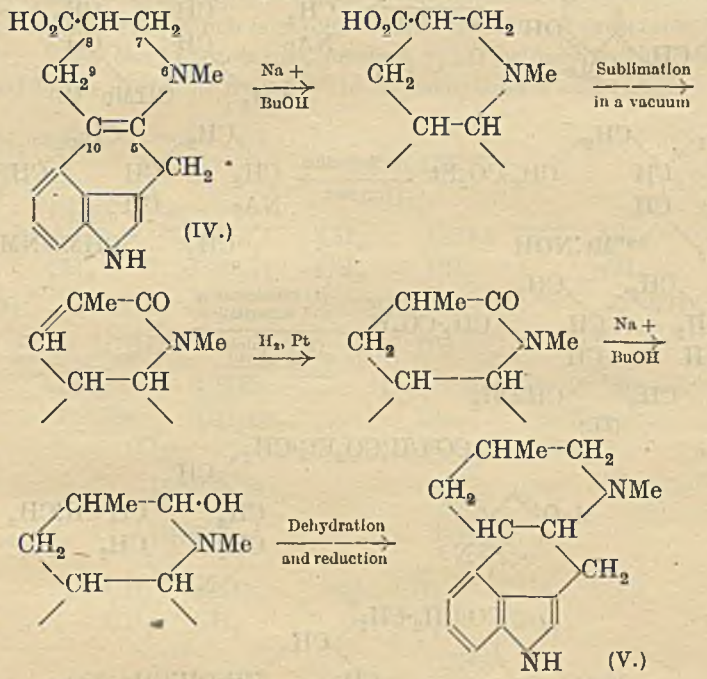
Ergot Alkaloids.[33]—Ergotoxine has been shown to be a mixture of ergocristine and two new alkaloids, ergocryptine and ergocornine. On degradation, these alkaloids yielded *l*-phenylalanine, *l*-leucine, and *l*-valine respectively; the remaining products, common to all three bases, were *d*-lysergic acid, *d*-proline, dimethylpyruvic acid [$CHMe_2{\cdot}CO{\cdot}CO_2H$ (III)] and ammonia, the alkaloids thus differing from the ergotamine group only in containing (III) in place of pyruvic acid.[34]

In continuation of work previously reported,[33] A. Stoll and A. Hofmann [35] have prepared the *d*- and the *l*-forms of lysergic and *iso*lysergic azides, and by reaction with a series of optically active β-amino-alcohols have prepared a

[30] *J. Org. Chem.*, 1942, **7**, 556.
[31] D. G. Harvey, E. J. Miller, and W. Robson, *J.*, 1941, 153.
[32] (Miss) A. Mookerjee, *J. Indian Chem. Soc.*, 1941, **18**, 33, 485; 1943, **20**, 11.
[33] *Ann. Reports*, 1939, **36**, 331.
[34] A. Stoll, A. Hofmann, and B. Becker, *Helv. Chim. Acta*, 1943, **26**, 1570, 1602.
[35] *Ibid.*, pp. 922, 929, 944.

variety of analogues of the ergobasine-ergobasinine type. The physiological activity of these products is more dependent on their configuration than on their constitution.

The conversion of *d*-lysergic acid (IV) into an optically active 6 : 8-dimethylergoline (V) has been improved; the degradation, which probably proceeds by the route shown, has been repeated with *dl*-lysergic acid, the *dl*-dimethylergoline thus obtained [36] being identical in all respects with the synthetic material.[37]

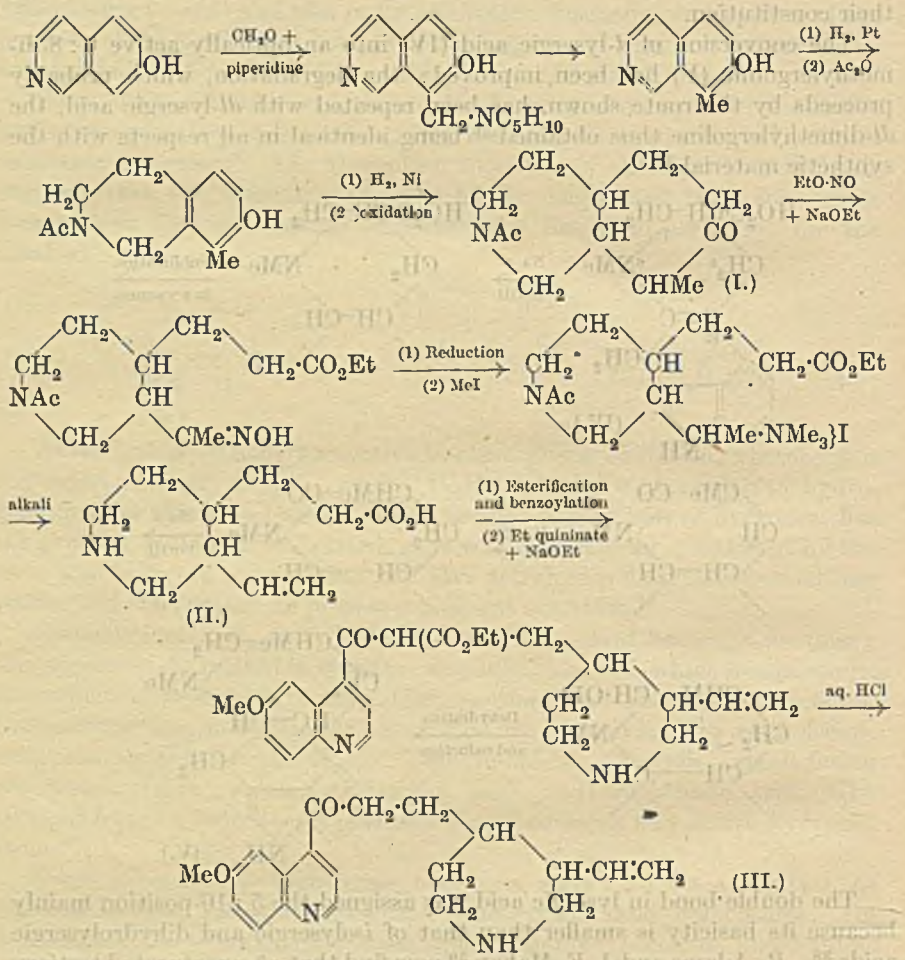


The double bond in lysergic acid was assigned the 5 : 10-position mainly because its basicity is smaller than that of *iso*lysergic and dihydrolysergic acids.[38] R. Adams and J. E. Mahan [39] now find that αβ-unsaturated tertiary amines possess higher basic dissociation constants than their saturated counterparts, and point out that the structure (IV) for lysergic acid therefore requires revision; probably the double bond occupies the 9 : 10-position in lysergic acid, and (IV) may correctly represent *iso*lysergic acid.

Cinchona Alkaloids.—R. B. Woodward and W. E. Doering [40] have announced the synthesis of *d*-quinotoxine (III); since the conversion of this substance into quinine was achieved in 1918 by P. Rabe,[41] the first total

[36] R. G. Gould, L. C. Craig, and W. A. Jacobs, *J. Biol. Chem.*, 1942, **145**, 487.
[37] W. A. Jacobs and L. C. Craig, *ibid.*, 1939, **130**, 399.
[38] L. C. Craig, T. Shedlovsky, R. G. Gould, and W. A. Jacobs, *ibid.*, 1938, **125**, 289.
[39] *J. Amer. Chem. Soc.*, 1942, **64**, 2588.
[40] *Ibid.*, 1944, **66**, 849. [41] *Ber.*, 1918, **51**, 466.

synthesis of quinine has been realised. Full details of the procedure are not yet available, but the method employed is outlined below :



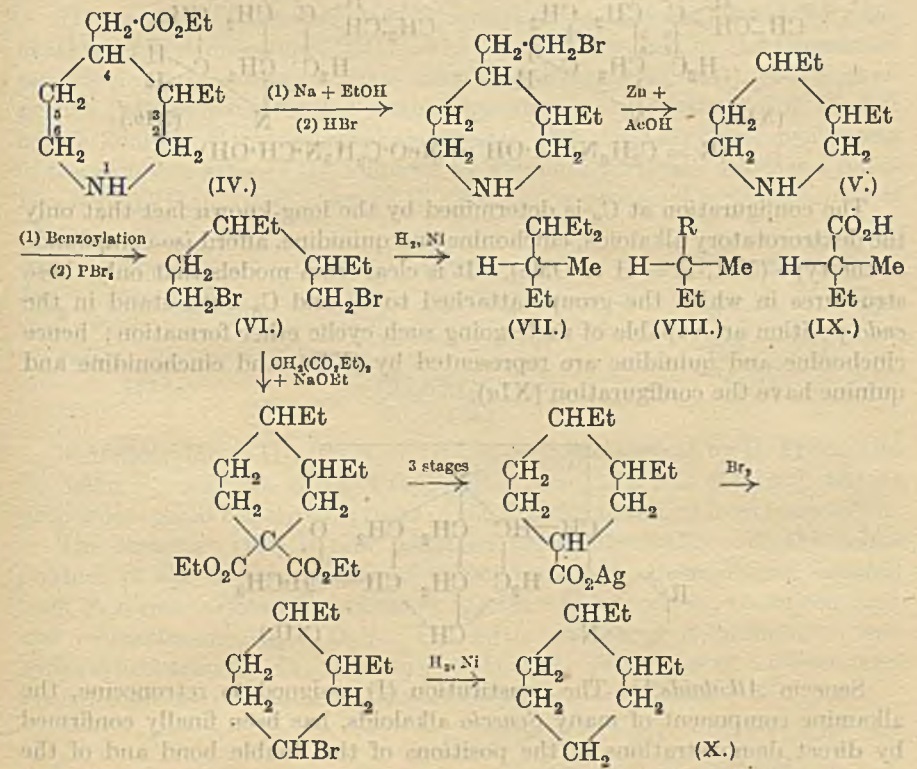
Two stereoisomeric forms of the ketone (I) were obtained, corresponding to *cis*- and *trans*-fusion of the rings; the *cis*-compound was employed for the succeeding stage. The conversion of *dl*-homomeroquinene (II) into *dl*-quinotoxine (III) was achieved by the methods developed by P. Rabe [42]; the same series of reactions has been carried out independently by M. Prostenik and V. Prelog,[43] starting with *d*-homomeroquinene obtained from natural cinchonine. Resolution of the synthetic quinotoxine (III) was achieved through the dibenzoyl-*d*-tartrate.

V. Prelog and E. Zalán [44] have determined the relative configurations of the asymmetric centres C_3, C_4, and C_8 of the cinchona alkaloids. It was

[42] *Ber.*, 1918, **51**, 1360; 1919, **52**, 1842. [43] *Helv. Chim. Acta*, 1943, **26**, 1965.
[44] *Ibid.*, 1944, **27**, 535, 545. Cf. J. Kenner, *Ann. Reports*, 1922, **19**, 156.

already known that all the alkaloids possess the same configuration at C_3 and C_4, since they all yield identical piperidine and quinuclidine derivatives on degradation; it was also known that cinchonidine differs from cinchonine (and similarly quinine from quinidine) in having the opposite configuration at both C_8 and C_9.[45]

(+)-Cincholoipon ethyl ester (IV), obtained from cinchonine, was degraded by the route shown to (−)-3-methyl-4-ethylhexane (VII), in which the sole remaining asymmetric centre is that of C_3 of the original alkaloid. The lævorotatory hydrocarbons of the type CHMeEtR (R>Et) are known to form a steric series, which is assigned the conventional projection formula (VIII).[46]. That the lævorotatory product (VII) belongs to this series was confirmed by its synthesis from (−)-methylethylacetic acid (IX).



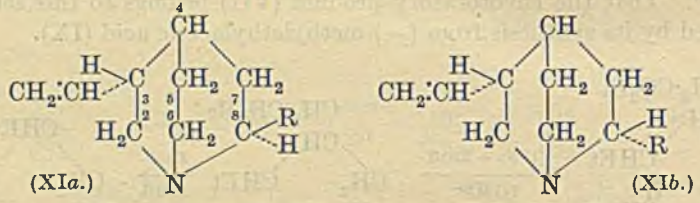
The configuration at C_4, relative to that at C_3, was decided by determining whether the substituents in the piperidine (V) stood in the *cis*- or *trans*-position. The derived dibromide (VI) was converted by a series of reactions, all of which proceeded under mild conditions and did not involve the asym-

[45] T. A. Henry, "The Plant Alkaloids," 3rd Edition, p. 423 (London, 1939).

[46] P. A. Levene and R. E. Marker, *J. Biol. Chem.*, 1931, **91**, 405, 761; **92**, 455; K. Freudenberg, "Stereochemie," pp. 679, 717 (Leipzig, 1932).

metric centres, into 1 : 2-diethyl*cyclo*hexane (X), which was optically inactive, and hence possessed the *cis*-configuration. This conclusion appears to receive independent support from the synthesis of quinotoxine referred to above, in which the *cis*-form of (I) gave rise to products having the correct configuration; it should, however, be pointed out that the evidence on which configurations were assigned to the two forms of (I) has not yet been published.

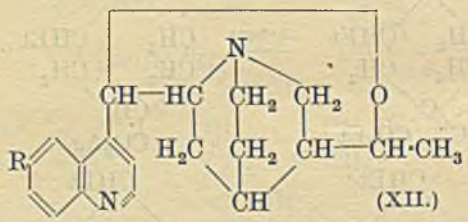
The establishment of the configurations at C_3 and C_4 limits the stereochemical possibilities for the cinchona alkaloids to the two structures (XI*a* and XI*b*).*



(XI*a*.) (XI*b*.)

(R = $C_9H_6N{\cdot}CH{\cdot}OH$ or $MeO{\cdot}C_9H_5N{\cdot}CH{\cdot}OH$)

The configuration at C_8 is determined by the long-known fact that only the dextrorotatory alkaloids, cinchonine and quinidine, afford *iso*-compounds of the type (XII, R = H or OMe). It is clear from models that only those structures in which the groups attached to C_3 and C_8 both stand in the *endo*-position are capable of undergoing such cyclic ether formation; hence cinchonine and quinidine are represented by (XI*b*), and cinchonidine and quinine have the configuration (XI*a*).



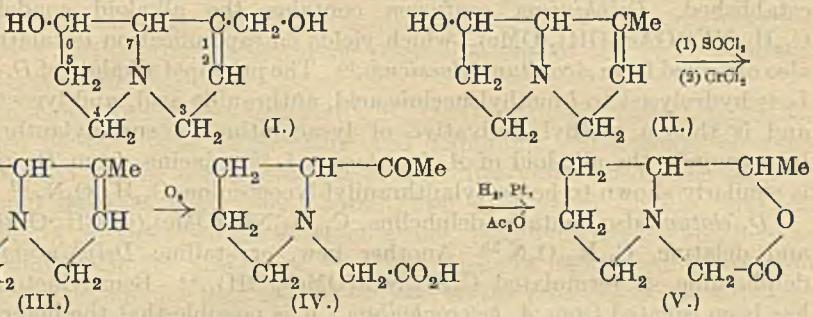
(XII.)

Senecio *Alkaloids*.[47]—The constitution (I) assigned to retronecine, the alkamine component of many *Senecio* alkaloids, has been finally confirmed by direct demonstrations of the positions of the double bond and of the secondary hydroxyl group. Deoxyretronecine (II) was converted, through the chloro-compound, into *iso*heliotridene (III), ozonolysis of the hydrochloride then producing a ketonic amino-acid (IV), which gave a positive iodoform test, and on reduction yielded a hydroxy-amino-acid, readily con-

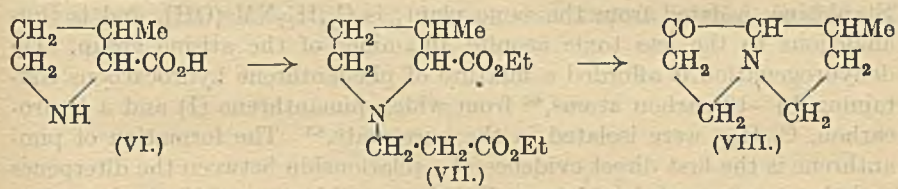
[47] *Ann. Reports*, 1942, **39**, 202.

* In these formulæ the conventions customary in the terpene series are employed, *i.e.*, C_5 and C_6 are in front of the plane of the paper, and dotted bonds extend behind that plane.

vertible into a basic lactone (V). These results can be interpreted only on the basis of the 1 : 2-position of the double bond.[48]

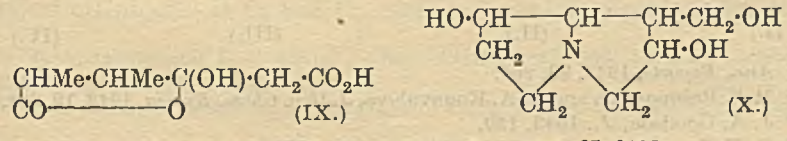


The position of the secondary hydroxyl group was established [49] by the synthesis of retronecanone (VIII), which is also obtained by the Oppenauer oxidation of retronecanol [the dihydro-derivative of (II)]. *l*-3-Methylproline (VI), prepared from 4-methylpiperidine by the method of E. Fischer and G. Zemplén,[50] was esterified and treated with ethyl acrylate; cyclisation of the resulting ester (VII), followed by ketonic hydrolysis, afforded *l*-retronecanone (VIII). Reduction of this substance produced a stereoisomer of retronecanol.



dl-Heliotridan (VIII, CH_2 for CO) has been synthesised by V. Prelog and E. Zalán [51]; only one of the two possible racemates was obtained, but its properties agreed closely with those of the *l*-isomer obtained from retronecine.

The structure (IX) is now preferred for monocrotalic acid, the acidic product of the hydrogenolysis of monocrotaline.[52] Rosmarinine, isolated from *S. rosmarinifolius* [47] and other species,[53] is hydrolysed to senecic acid and rosmarinecine, $C_8H_{15}O_3N$. The latter substance is probably a trihydroxyheliotridan; since it does not show the properties of a ψ-base, and is unaffected by periodic acid, the structure (X) is advanced.[53]



[48] R. Adams and J. E. Mahan, *J. Amer. Chem. Soc.*, 1943, **65**, 2009.
[49] R. Adams and N. J. Leonard, *ibid.*, 1944, **66**, 257.
[50] *Ber.*, 1909, **42**, 2989. [51] *Helv. Chim. Acta*, 1944, **27**, 531.
[52] R. Adams and J. M. Wilkinson, *J. Amer. Chem. Soc.*, 1943, **65**, 2203; cf. R. Adams and R. S. Long, *ibid.*, 1940, **62**, 2289.
[53] (Miss) M. F. Richardson and F. L. Warren, *J.*, 1943, 452.

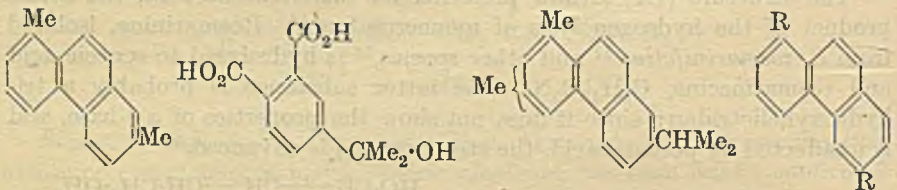
Aconitum *and* Delphinium *Alkaloids*.[54]—The long-suspected structural relationship between the alkaloids of these two genera has now been firmly established. *Delphinium confusum* contains the alkaloid condelphine, $C_{19}H_{23}NEt(OAc)(OH)_2(OMe)_2$, which yields on saponification *iso*talatisidine, also obtained from *Aconitum talassicum*.[55] The principal alkaloid of *D. elatum* L. is hydrolysed to *l*-methylsuccinic acid, anthranilic acid, and lycoctonine, and is thus a methyl derivative of lycaconitine (= succinylanthranilyl-lycoctonine), the alkaloid of *A. lycoctonum* L.[56]; ajacine, from *D. ajacis*,[57] is similarly shown to be acetylanthranilyl-lycoctonine, $C_{34}H_{46}O_9N_2$.[58]

D. elatum also contains delpheline, $C_{19}H_{22}NEt(OMe)_3(\cdot O\cdot CH_2\cdot O\cdot)(OH)$,[59] and delatine, $C_{19}H_{25}O_3N$.[58] Another new, crystalline *Delphinium* base, delphamine, is formulated $C_{20}H_{23}NEt(OMe)_3(OH)_4$.[55] Benzoylheteratisine has been isolated from *A. heterophyllum*; it is possible that the heteratisine previously isolated from this source is an artefact arising by hydrolysis of the benzoyl derivative during the isolation process.[60]

Delphinine, the principal crystalline alkaloid of *D. staphisagria*, now shown to be $C_{33}H_{45}O_9N$, exhibits a close parallelism with aconitine in its reactions, and in its partial structural formula [61]:

Delphinine $C_{19}H_{21}NMe(OMe)_4(OAc)(OBz)(OH)$
Aconitine $C_{19}H_{19}NEt(OMe)_4(OAc)(OBz)(OH)_3$.

Staphisine, isolated from the same plant, is $C_{21}H_{27}NMe(OH)$, and is thus analogous to the less toxic aconite alkamines of the atisine group. On dehydrogenation it afforded a mixture of phenanthrene hydrocarbons containing 16—19 carbon atoms,[62] from which pimanthrene (I) and a hydrocarbon, $C_{19}H_{20}$, were isolated in the pure state.[63] The formation of pimanthrene is the first direct evidence of a relationship between the diterpenes and the aconite and delphinium alkamines. Oxidation of the hydrocarbon $C_{19}H_{20}$ yielded first a quinone, and then an acid, $C_{11}H_{12}O_5$, possibly α-hydroxy*iso*propylphthalic acid (II), and it is suggested that the hydrocarbon may be a methylretene (III).

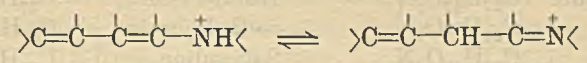


(I.) (II.) (III.) (IV.)

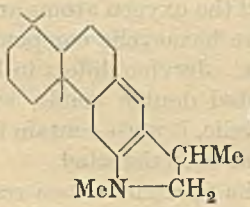
[54] *Ann. Reports*, 1942, **39**, 206.
[55] M. S. Rabinovitch and R. A. Konovalova, *J. Gen. Chem. Russia*, 1942, **12**, 321, 329.
[56] J. A. Goodson, *J.*, 1943, 139.
[57] O. Keller and O. Volker, *Arch. Pharm.*, 1913, **251**, 209.
[58] J. A. Goodson, *J.*, 1944, 108; cf. M. V. Hunter, *Pharm. J.*, 1943, **150**, 82.
[59] J. A. Goodson, *J.*, 1944, 665.
[60] W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, 1943, **147**, 571; 1942, **143**, 605.
[61] *Idem, ibid.*, 1939, **127**, 361; **128**, 431; 1940, **136**, 303.
[62] *Idem, ibid.*, 1941, **141**, 67. [63] *Idem, ibid.*, 1944, **152**, 645.

The hydrocarbon, $C_{17}H_{16}$, obtained from the dehydrogenation of atisine,[64] afforded a mixture of acids on oxidation; although these products have not been unequivocally identified, their nature suggests that the C_{17} hydrocarbon may be a methylethylphenanthrene possessing one of the two structures (IV; R, R = Me, Et).

Although the alkamines aconine ($C_{25}H_{41}O_9N$) and delphonine ($C_{24}H_{39}O_7N$), obtained by hydrolysis of aconitine and delphinine respectively, show no unsaturation by the usual tests, the strong ultra-violet absorption of their solutions indicates that they contain two conjugated double linkages, probably in proximity to the nitrogen atom; tetrahydroatisine and heteratisine show a similar absorption.[65] The unusually high basic dissociation constant of delphonine may also be ascribed to the presence of the structure $C=C-N$:[39, 65]; the lower ultra-violet absorption shown by the salts of these bases would then be due to the existence of an equilibrium of the type :



The presence of these double bonds makes possible a tetracyclic structure for the alkaloids, which is more closely in harmony with their apparent relationship to the diterpenoids. The structure (V) has been put forward as an example of a possible type of fundamental nucleus for the alkaloids of this group.[65]



(V.)

Veratrum *and* Solanum *Alkaloids*.[66]—Selenium dehydrogenation of solanidine affords, in addition to Diels's hydrocarbon,[67] 5-methyl-2-ethylpyridine,[68] suggesting the presence of a six-membered nitrogenous ring, and the structure (I) has been advanced. By employing methods familiar in the steroid field, V. Prelog and S. Szpilfogel [69] have converted solanidine into all four possible solanidanols (dihydrosolanidines), differing in their stereochemical orientation at C_3 and C_5, and also into *allo*solanidane (rings A/B

[64] W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, 1942, **143**, 589; A. Lawson and J. E. C. Topps, *J.*, 1937, 1640.

[65] L. C. Craig, L. Michaelis, S. Granick, and W. A. Jacobs, *J. Biol. Chem.*, 1944, **154**, 293.

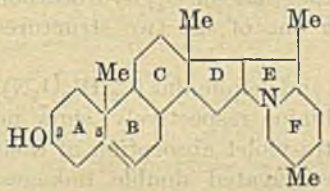
[66] *Ann. Reports*, 1940, **37**, 378; 1942, **39**, 207.

[67] H. Soltyz and K. Wallenfels, *Ber.*, 1936, **69**, 811; H. Rochelmeyer, *Arch. Pharm.*, 1936, **274**, 543.

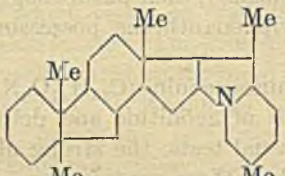
[68] V. Prelog and S. Szpilfogel, *Helv. Chim. Acta*, 1942, **25**, 1306; W. A. Jacobs and L. C. Craig, *Science*, 1943, **97**, 122; *J. Biol. Chem.*, 1943, **149**, 271.

[69] *Helv. Chim. Acta*, 1944, **27**, 390.

cis). There is a very close parallelism between the optical rotations of these products and those of the corresponding cholesterol derivatives.



(I.)



(II.)

$HO_2C{\cdot}CH_2{\cdot}CMe{\cdot}CO_2H$
$HO_2C{\cdot}CH_2{\cdot}CMe{\cdot}CO_2H$
(III.)

The formulæ of several alkaloids of the veratrine group have been revised, and all the alkamines are now found to be C_{27} compounds, thus strengthening the formal relationship with the *Solanum* group.[70, 71]

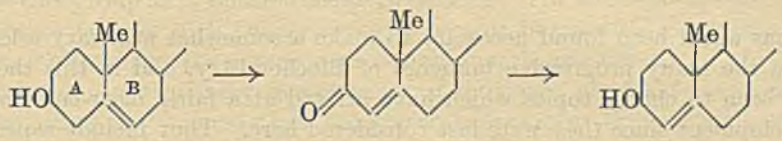
Protoverine	$C_{27}H_{43}O_9N$	Rubijervine	$C_{27}H_{43}O_2N$
Cevine	$C_{27}H_{43}O_8N$	*iso*Rubijervine	$C_{27}H_{43}O_2N$
Germine	$C_{27}H_{43}O_8N$	Solasodine	$C_{27}H_{43}O_2N$
Jervine	$C_{27}H_{39}O_3N$	Solanidine	$C_{27}H_{43}ON$

W. A. Jacobs and L. C. Craig [71] have discussed the constitution of these alkaloids in the light of recent experimental results. With the exception of jervine, all these substances are tertiary bases containing one ethylenic linkage, and in each case all the oxygen atoms are accounted for as hydroxyl groups. They are therefore hexacyclic compounds, and have the nitrogen atom common to two rings. Jervine differs in being a secondary base, and in containing two conjugated double bonds, which can be hydrogenated. Since it is probably pentacyclic, it must contain two further, resistant double bonds, which have not so far been detected.

On dehydrogenation, the veratrine bases resemble solanidine in giving 5-methyl-2-ethylpyridine, but differ in that they do not yield Diels's hydrocarbon. Instead, the more highly oxygenated bases afford cevanthridine, and a mixture of hydrocarbons which appear to be tetracyclic or pentacyclic fluorene derivatives; rubijervine, on the other hand, affords a hydrocarbon, $C_{18}H_{16}$, similar to, but not identical with, 3′-methyl*cyclo*pentenophenanthrene; its properties are in fairly close agreement with those of the 1′-methyl isomer. Moreover, *iso*rubijervine readily forms a digitonide, and rubijervine does so rather more slowly. The failure of the other veratrine bases to give precipitates with digitonin may be due either to their higher hydroxyl content or to the absence of the necessary steroidal structure. Rubijervine has been converted,[72] through the related ketone rubijervone, into an isomer, probably a mixture of *allo*rubijervine and *epiallo*rubijervine; this product reacted only very slowly with digitonin, and differed from the original material in giving a strong Rosenheim reaction. It is concluded

[70] W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, 1943, **148**, 41, 51, 57; **149**, 271.
[71] *Idem*, *ibid.*, 1943, **149**, 451. [72] *Idem*, *ibid.*, 1944, **152**, 641.

that the allomerisation proceeded in a manner analogous to the corresponding conversion of cholesterol into *allo*cholesterol :



Rubijervine accordingly cannot possess an angular methyl group at C_5, and therefore cannot contain the skeleton (II) previously proposed for the veratrine alkaloids, but is probably a hydroxy-derivative of (I). Attempts to achieve a similar allomerisation of cevine were unsuccessful.

The authors consider the possibility that the fluorene hydrocarbons obtained by dehydrogenation of cevine, germine, and protoveratrine may arise by a rearrangement, or by a recyclisation of the side chain (formed from rings E and F) on to C_{16}. If such were the case, a steroid structure similar to (I) could be assigned to all the veratrine alkaloids; however, it is difficult to explain, on such a basis, the production, in high yield, of the acid (III), by oxidation of cevine and germine (but not rubijervine), unless this also involves a rearrangement. The nature of ring B in these alkaloids thus requires further investigation. Surface film studies [73] indicate the presence of an extended, hexacyclic system and are consistent with structures of the types (I) and (II). H. T. O.

[73] A. Rothen and L. C. Craig, *J. Amer. Chem. Soc.*, 1943, **65**, 1102.

BIOCHEMISTRY.

It has again been found necessary to make a somewhat arbitrary selection from the many progressive branches of Biochemistry, and in this the aim has been to choose topics which have arrived at a fairly clear-cut stage of development since they were last considered here. They include aspects of metabolism, hormones, nutrition, and chemotherapy.

I. Phosphorylation Mechanisms.

Phosphate Bond Energy.

The inter-relationships of phosphorylations in the transport and storage of metabolic energy [1] have been further clarified and their range and interpretation have been extended.[2, 3, 4] It is doubtful if under physiological conditions appreciable synthesis of phosphoric esters could occur by reversal of their hydrolytic (esterase) cleavage, and the primary introduction of phosphoric groups falls into two main classes, though doubtless other routes [5, 6] also exist.

Phosphorolysis of the glucosidic type of linkage in poly- or di-saccharides occurs reversibly, as is described below, and thus provides for the introduction of phosphate into organic metabolites without the need for external energy sources. The second type of phosphorylation is associated with a marked increase of free energy, which is commonly derived from enzymic dehydrogenation accompanying the addition of phosphate to a double bond.

The differences in energy level in these two types of compound are naturally reflected in their widely different properties. The esters of phosphoric acid with alcohols such as hexoses (including the " glucosidic " type represented by glucose 1-phosphate), pentoses, trioses, glycerol, choline, serine, or the 2- or 3-position in glyceric acid, are stable compounds; their hydrolysis whether by acids or enzymes is reversible, and is accompanied by relatively small energy change (ΔF about -3000 cals.). But the cleavage of the second type of phosphoric compound is strongly exothermic (ΔF about $-11{,}000$ cals.). Lipmann [3] has therefore introduced for the latter the symbol $\sim$ P, denoting the " high energy phosphate bond " by means of which the high potential energy of the phosphorus linkage may be indicated: the phosphate group he writes $\sim$ ph. From the biochemical standpoint this is justified by the emphasis which it places on the ability of such high potential groups to promote synthetic reactions. Examples of this type of compound include anhydrides formed from phosphoric acid, with an organic

[1] See *Ann. Reports*, 1941, **38**, 241; 1940, **37**, 386, 417.

[2] H. M. Kalckar, *Chem. Rev.*, 1941, **28**, 71; *Biol. Rev.*, 1941, **17**, 28.

[3] F. Lipmann, " Advances in Enzymology," Interscience Press, N.Y., 1941, **1**, 99.

[4] A. A. Green and S. P. Colowick, *Ann. Rev. Biochem.*, 1944, **13**, 155.

[5] H. M. Kalckar, *Biochem. J.*, 1939, **33**, 631.

[6] S. P. Colowick, M. S. Welch, and C. F. Cori, *J. Biol. Chem.*, 1940, **133**, 359; 1941, **137**, 343.

phosphate as in the two terminal groups of adenosine triphosphate, or with a carboxyl as in 1 : 3-diphosphoglyceric acid or acetyl phosphate, or with an acidic enol group as in phospho*enol*pyruvic acid. The amidophosphate bond is another type of high energy linkage, present in creatine phosphate and arginine phosphate, the energy reservoirs of muscle and nerve.

Following the primary introduction of inorganic phosphate into metabolites, other enzymic reactions are known to transport these groups, either inter- or intra-molecularly, thus forming the wide variety of phosphorylated intermediates. According to the energy changes involved, such reactions may be reversible or irreversible. Thus reversible interchange of phosphate occurs, on the higher energy level, between the adenosine triphosphate–adenylic acid system and the creatine–creatine phosphate or phosphoglycerate–diphosphoglycerate systems. On the lower potential level it may be between glucose 1-phosphate and glucose 6-phosphate (enzyme, phosphoglucomutase), or between 3- and 2-phosphoglyceric acids (phosphoglyceromutase). The phosphorylation of glucose to glucose 6-phosphate by adenosine triphosphate (hexokinase), *i.e.*, a change from the high to low level, is irreversible. The concept of phosphate bond energy, and in a wider sense of group potential, has many applications to biological syntheses, including acetylations, methylations, and aminations (cf. 3). It plays an important part in animal phosphorylations, as will be briefly considered here.

Primary Introduction of Phosphate Groups : Phosphorylase.

The preparation from skeletal muscle of this enzyme, which esterifies a glucose unit of a polysaccharide, or conversely synthesises polysaccharide from glucose 1-phosphate, is fully described by Cori and co-workers.[7, 8, 9, 10] It has been obtained in a crystalline (*a*) and an amorphous (*b*) form. The former is a euglobulin of M.W. 340,000—400,000 and has 60—70% of its maximum activity without addition of adenylic acid. The more soluble *b* form is inactive without addition of adenylic acid, but both *a* and *b* are equally active in its presence. The optimum rate of conversion is 4×10^4 mols. of glucose 1-phosphate to glycogen/mol. enzyme/min. at 30°. Glucose competitively inhibits the activity, while cysteine increases both activity and solubility. Extracts of muscle and spleen contain an enzyme (" PR ") which, like trypsin, removes the prosthetic group from *a*, converting it into the amorphous *b* form. Simultaneously, pentose (0·3 μg./mg. of protein) is lost, but the substance split off is not adenylic acid. Added adenylic acid does not render the *b* form crystallisable, nor is it firmly bound as is that present in *a*. Possibly this non-dissociable union of the prosthetic group is a protection *in vivo*.

As with vegetable phosphorylase [11] the equilibrium position varies with

[7] A. A. Green and G. T. Cori, *J. Biol. Chem.*, 1943, **151**, 21.
[8] G. T. Cori and A. A. Green, *ibid.*, p. 31.
[9] C. F. Cori, G. T. Cori, and A. A. Green, *ibid.*, p. 39.
[10] G. T. Cori and C. F. Cori, *ibid.*, p. 57.
[11] C. S. Hanes and E. J. Marshall, *Biochem. J.*, 1942, **36**, 76.

pH; polysaccharide is formed from Cori ester only when a little " priming " polysaccharide is added. The reaction is considered to be [9]: glucose 1-phosphate + terminal glucose unit $\rightleftharpoons$ maltosidic chain unit + inorganic phosphate. The terminal glucose units are supplied by the end groups of the highly branched glycogen or amylopectin molecule (starch amylose does not activate animal phosphorylase [10]), polysaccharide synthesis consisting of a lengthening of the side chains by addition of glucose units in 1 : 4-glucosidic linkages [9, 12, 13] to form long unbranched chains of glucopyranose units.

When a supplementary enzyme from heart or liver, obtained free from phosphorylase, accompanies crystalline phosphorylase, a branched-chain type of polysaccharide resembling glycogen results. Presumably branched-chain polysaccharides such as glycogen and amylopectin arise from the joint action of phosphorylase and another enzyme [10] or factor.[12] It is uncertain whether the supplementary enzyme in Cori's experiments is another type of phosphorylase, able to establish 1 : 6-glucosidic linkages, or else some kind of diastase not identical with that of blood serum.[10]

The yield of phosphorylase from rabbit skeletal muscle (40—80 mg./100 g.) is not altered by previous stimulation of the muscle, but the proportion crystallisable is diminished.[8, 14] Phosphorylase occurs in a variety of tissues, and in embryonic tissues is related to the activity of their glycogen metabolism.[14, 15] It is contained in adipose tissue,[15] which utilises glycogen,[15] and in cartilage the enzyme may produce phosphoric esters, yielding phosphate needed for calcification.[16]

Adenylic acid is not a component of potato phosphorylase [17] or of disaccharide phosphorylases,[18] which require no coenzyme. It is noteworthy that in muscle phosphorylase adenylic acid acts as coenzyme without any evidence of its phosphorylation.[19] Adenosine di- or tri-phosphates have no, and inosic acid only feeble, coenzyme activity.[9, 20]

Adenosine Triphosphatase.

The energy liberated by hydrolysis of the final [21] phosphoric group of adenosine triphosphate (ATP) is believed to be directly utilised for muscular contraction.[22] Engelhardt's important discovery in 1939 [22] that myosin

[12] W. N. Haworth, S. Peat, and E. J. Bourne, *Nature*, 1944, **154**, 236.

[13] W. N. Haworth, R. L. Heath, and S. Peat, *J.*, 1942, 55; W. Z. Hassid, G. T. Cori, and R. M. McCready, *J. Biol. Chem.*, 1943, **148**, 89; K. H. Meyer, " Advances in Enzymology," 1943, **3**, 109; W. Z. Hassid, *Ann. Rev. Biochem.*, 1944, **13**, 59.

[14] A. Mirski and E. Wertheimer, *Biochem. J.*, 1942, **36**, 221.

[15] B. Shapiro and E. Wertheimer, *Biochem. J.*, 1943, **37**, 397; A. Mirski, *ibid.*, 1942, **36**, 232; E. Wertheimer, *Nature*, 1943, **152**, 565.

[16] A. B. Gutman and E. B. Gutman, *Proc. Soc. Exp. Biol. Med.*, 1941, **48**, 687; A. B. Gutman, F. B. Warrick, and E. B. Gutman, *Science*, 1942, **95**, 461.

[17] D. E. Green and P. K. Stumpf, *J. Biol. Chem.*, 1942, **142**, 355.

[18] M. Doudoroff, *ibid.*, 1943, **151**, 351; P. H. Hidy and H. G. Day, *ibid.*, 1944, **152**, 477.

[19] Cf. ref. (3), p. 124.

[20] C. F. Cori, *Cold Spring Harbor Symposia on Quantitative Biology*, 1939, **7**, 260.

[21] Cf. refs. (32), (33), and (28). [22] See *Ann. Reports*, 1941, **38**, 241.

" which is the contractile constituent of muscle is at the same time the catalytic agent which promotes the chemical reaction which provides the direct source of energy of muscular activity " [23] has been widely accepted as probable after several careful studies,[22, 21, 25, 26] and so far no more active fraction has been isolated from this globulin. Nevertheless, cataphoresis [25] and sedimentation [26] analyses suggest that myosin may not be quite homogeneous, though nearly so.[27] The most serious challenge to the view that myosin itself is the enzyme is the recent demonstration by Kalckar [28] that an adenosine polyphosphatase, present in the soluble albumin fraction from potato and 50—100 times more active than myosin, is strongly and apparently somewhat specifically adsorbed by myosin. But even if the activity of myosin should eventually prove to be due to adsorbed adenosine triphosphatase, the latter might still be linked to contraction of myosin. This potato enzyme, like that from liver, hydrolyses ATP directly to adenylic acid without intervention of myokinase. It thus differs from the muscle enzyme, which is considered specific for the triphosphate inasmuch as it does not act upon adenosine diphosphate (ADP) except through myokinase;[28] but it attacks inosine triphosphate even faster than ATP.[29]

Although iodoacetic acid does not inactivate adenosine triphosphatase,[30] oxidation does so, and SH compounds reverse the inactivation.[31] Apparently the establishment of the single thioether linkages by the former reagent is to be distinguished from the cross-linked S–S bonds believed to be here produced by oxidants.

Radioactive phosphorus has been used to show in muscle the coupling of oxidation with the phosphorylation of adenylic acid and creatine [32] and the rapidity of resynthesis of ATP after its breakdown.[33, 34]

Phosphokinases (*Phospherases*).

Myokinase.—Although Lipmann's terminology does not differentiate them, the terminal phosphate group of ATP is more reactive than that of the diphosphate (ADP), the latter being unable to transfer phosphate directly (*e.g.*, to glucose in presence of hexokinase), but requiring the presence of a water-soluble enzyme, myokinase, obtained from muscle and other tissues.[35, 36] This enzyme, which is stable to heat and to acid, catalyses the reversible

[23] W. A. Engelhardt, *Yale J. Biol. Med.*, 1942, **15**, 21 (Engl. trans. from Russian orig.).
[24] D. M. Needham, *Biochem. J.*, 1942, **36**, 113.
[25] K. Bailey, *ibid.*, p. 129.
[26] G. Schramm and H. H. Weber, *Kolloid-Z.*, 1942, **100**, 242; Brit. Chem. Physiol. Abs., 1943, III, 348.
[27] M. Ziff and D. H. Morre, *J. Biol. Chem.*, 1944, **153**, 653.
[28] H. M. Kalckar, *ibid.*, p. 355. [29] A. Kleinzeller, *Biochem. J.*, 1942, **36**, 729.
[30] D. M. Needham, *ibid.*, p. 113. [31] M. Ziff, *J. Biol. Chem.*, 1944, **153**, 25.
[32] R. F. Furchgott and E. Shorr, *ibid.*, 1943, **151**, 65.
[33] E. V. Flock and J. L. Bollman, *ibid.*, 1944, **152**, 371.
[34] H. M. Kalckar, J. Dehlinger, and A. Mehler, *ibid.*, 1944, **154**, 275.
[35] S. P. Colowick and H. M. Kalckar, *ibid.*, 1943, **148**, 117.
[36] H. M. Kalckar, *ibid.*, p. 127.

reaction : 2ADP $\rightleftharpoons$ ATP + adenylic acid. Inosin diphosphate is not affected.[29] The enzyme is inactivated by oxidants and activated by SH compounds, and it is capable of transferring 4 times its own weight of phosphorus per min. at 30°.

Hexokinase.—The occurrence of the hexokinase reaction : hexose + ATP $\longrightarrow$ hexose 6-phosphate + ADP, is probable in various cells which metabolise glucose, and from several of these this water-soluble enzyme has been extracted.[35, 37, 38] With yeast hexokinase direct phosphorylation of the 6-position of the hexose occurs with glucose or fructose,[39] but it is possible that in aerobic liver suspensions fructose may be directly phosphorylated in position 1, or alternatively, as has been suggested for galactose 1-phosphate,[40] there may be an equilibrium between these 1-phosphates and Cori ester.[39]

Hexokinase is of special importance in the synthesis of glycogen from glucose, the glucose 6-phosphate being reversibly converted *via* the 1-phosphate into glycogen, by means of the enzymes phosphoglucomutase and phosphorylase (cf. 37).

Phosphorylation of Fructose 6-Phosphate.—This reaction proceeds by way of an enzyme not yet isolated, sometimes called Neuberg ester phospherase. It catalyses the reaction : fructose 6-phosphate + ATP $\longrightarrow$ fructose 1 : 6-diphosphate + ADP. It is stated to be inhibited by oxidising agents and even by O/R indicators of $E_0 > 0{\cdot}05$ v., and this sensitivity has been held to be the mechanism of the Pasteur effect, by which the fermentation is checked aerobically [41]; but this is perhaps an over-simplification.[42]

Other Enzymes concerned in Reactions of Phosphorylated Intermediates.

Recent outstanding advances include the purification of phosphoglucomutase,[37] the isolation of aldolase (or zymohexase) of muscle,[43] now crystallised and its distribution studied,[44] and of enolase (crystalline mercury salt).[45] It is stated that the addition of phosphate to 3-phosphoglyceraldehyde is non-enzymic, and that the unknown intermediate in the dehydrogenation of this triose phosphate (previously considered to be 1 : 3-diphosphoglyceraldehyde[22]) has the nature of a " loose physical addition product," analogies for which are suggested.[46]

Oxidative Phosphorylations.

Preceding the formation of pyruvate. The reaction just mentioned was the first in which the oxidative formation of high energy phosphate bonds

[37] S. P. Colowick and E. W. Sutherland, *J. Biol. Chem.*, 1942, **144**, 423.
[38] I. Huzák, *Biochem. Z.*, 1942, **312**, 315.
[39] C. F. Cori, *Biological Symposia*, 1941, **5**, 131.
[40] H. W. Kosterlitz, *Biochem. J.*, 1943, **37**, 318, 321, 322.
[41] W. A. Engelhardt and N. E. Sakov, *Biochimia*, 1943, **8**, 9.
[42] Cf. E. S. G. Barron, " Advances in Enzymology," 1943, **3**, 149 (p. 183).
[43] D. Herbert, H. Gordon, V. Subrahmanyan, and D. E. Green, *Biochem. J.*, 1940, **34**, 1108.
[44] O. Warburg and W. Christian, *Biochem. Z.*, 1943, **314**, 149, 399.
[45] *Idem*, *ibid.*, 1941—2, **310**, 384.
[46] O. Meyerhof and R. Junowicz-Kocholaty, *J. Biol. Chem.*, 1943, **149**, 71.

was clearly demonstrated, inorganic phosphate being incorporated into the carboxyl of the final product, 1 : 3-diphosphoglyceric acid. This is the more common mechanism by which such bonds arise. An alternative is seen in the action of enolase, which merely by the removal of a molecule of water from 2-phosphoglyceric acid reversibly produces the high energy enolic bond in the resulting phospho*enol*pyruvic acid : in this remarkable reaction the considerable energy of dehydration is conserved within the molecule (cf. 3).

Thus in the passage from a glucose unit of glycogen to pyruvate, through the well-known series of phosphorylated intermediates, one externally introduced ~ ph (from ATP) is required in the formation of fructose 1 : 6-diphosphate, and, since two mols. of pyruvate are formed, 2 ~ ph arise at each of the stages resulting in 1 : 3-diphosphoglyceric and phospho*enol*pyruvic acids. Thus the removal of 4 hydrogen atoms should yield a balance of 3 ~ ph per mol. of hexose metabolised. Starting from glucose, instead of glycogen, the primary phosphorylation by ATP and hexokinase consumes a further ~ ph, and at the pyruvate stage only 2 ~ ph remain on balance. Possibly in intact cells, as distinct from extracts, economies are effected by unknown mechanisms (*e.g.*, the formation of fructose 1 : 6-diphosphate could theoretically occur by intermolecular transfer of the 1-phosphate from Cori ester to fructose 6-phosphate).[3] The synthesis in intact cells of ATP from inorganic phosphate during glucose fermentation has been demonstrated.[47]

During pyruvate metabolism. The simpler conditions prevailing in bacterial extracts enabled Lipmann [48, 49, 50] to demonstrate the production of high energy phosphate bonds during the bacterial oxidation of pyruvate to acetate and carbon dioxide, and the formation of acetyl phosphate, now isolated as the pure silver salt.[50] The latter has been synthesised from monosilver phosphate and acetyl chloride and the properties and determination of monoacetyl phosphate are described.[51] It is assumed [3, 52] that in the enzymatic synthesis [50] the addition of phosphoric acid to the carbonyl group of pyruvic acid is followed by the dehydrogenation of the resulting (unknown) compound; the analogy with bisulphite and cyanohydrin compounds is suggested. This mechanism finds some support in purely chemical studies of E. Baer.[53, 54] The reaction is represented as a dehydrogenative decarboxylation : $CH_3{\cdot}CO{\cdot}CO_2H + HO{\cdot}ph \rightleftharpoons CH_3{\cdot}C(OH)(O{\cdot}ph){\cdot}CO_2H \xrightarrow{-2H} CH_3{\cdot}CO{\cdot}O \sim ph + CO_2$. The closely similar reaction occurring in cell-free extracts of *Esch. coli* : pyruvic acid + $H_3PO_4 \rightleftharpoons$ acetyl phosphate + formic

[47] D. J. O'Kane and W. W. Umbreit, *J. Biol. Chem.*, 1942, **142**, 25.
[48] Cf. *Ann. Reports*, 1940, **37**, 417.
[49] F. Lipmann, *J. Biol. Chem.*, 1940, **134**, 463; Symposium on Respiratory Enzymes, Univ. of Wisconsin Press, 1941, 145; *Federation Proc.*, 1942, **1**, 122.
[50] F. Lipmann, *J. Biol. Chem.*, 1944, **155**, 55.
[51] F. Lipmann and L. C. Tuttle, *ibid.*, 1944, **153**, 571.
[52] F. Lipmann, *Ann. Rev. Biochem.*, 1943, **12**, 1.
[53] *J. Amer. Chem. Soc.*, 1940, **62**, 1597.
[54] *J. Biol. Chem.*, 1942, **146**, 391.

acid,[55] has now been shown to be reversible,[56, 57] ^{13}C of radioactive formic acid appearing in the carboxyl group of the keto-acid. Since in the bacteria, though not in these cell-free extracts, carbon dioxide is normally in equilibrium with formic acid, the mechanism of a new method of carbon dioxide fixation into the carboxyl of pyruvic acid is revealed by these experiments. The synthetic reaction resulting in carbon dioxide fixation is able to proceed only because the dehydrogenation product is acetyl phosphate, and not the free acid: the formation of acetic acid from pyruvate would result in an energy loss of some 15,000 cals.[57]

As yet the evidence of the formation of acetyl phosphate or homologous compounds in animal tissues is indirect, based on reactions such as acetylations *in vivo*,[58] in isolated tissues [59] or in tissue extracts.[60] However, the oxidative formation of energy-rich phosphate bonds in such material has been repeatedly proved by the phosphorylation of adenylic acid, creatine, or glucose 6-phosphate, for example.[5, 32, 33, 61]

The efficiency of this process is unexpectedly high. The oxidation by a cytochrome system of α-ketoglutarate to succinate [62] causes esterification of 3 atoms of phosphorus for each oxygen atom consumed: in this 4-C dicarboxylic acids did not function as hydrogen carriers. Obviously an oxidative decarboxylation as formulated above could give only a 1 : 1 ratio. Even the highly speculative assumption of a diphosphate formation leaves a deficiency of one phosphorus atom esterified. Perhaps even more remarkable is the observation [63] that the whole pyruvic molecule is oxidised in heart extract with precisely the same efficiency; P/O ratio = 3. This indicates that no less than 15 high-energy phosphate bonds are established by the oxidation of 1 mol. of pyruvate, which at the level of 11,000 cals./bond shows the efficiency of conversion of oxidation into phosphate bond energy to be nearly 60%. If the course of pyruvate oxidation through the "tricarboxylic acid cycle" [61] be accepted, those of the five dehydrogenation reactions involved which have been shown to be accompanied by phosphorylation are as follows: α-Keto-acid oxidation (occurring twice) could generate $2 \times 3 \sim$ ph [62]; succinate ⟶ fumarate not more than 1 ~ph [61, 63]; malate to oxalo-

[55] M. Silverman and C. H. Werkman, *Proc. Soc. Exp. Biol. Med.*, 1940, **43**, 777; M. F. Utter and C. H. Workman, *Arch. Biochem.*, 1943, **2**, 491.

[56] M. F. Utter, C. H. Werkman, and F. Lipmann, *J. Biol. Chem.*, 1944, **154**, 723.

[57] F. Lipmann and L. C. Tuttle, *ibid.*, p. 725.

[58] E. A. Doisy, jun., and W. W. Westerfield, *ibid.*, 1943, **149**, 229; G. J. Martin and E. H. Rennenbaum, *ibid.*, 1943, **151**, 417; K. Block and D. Rittenberg, *ibid.*, 1944, **155**, 243.

[59] P. J. G. Mann, M. Tennenbaum, and J. H. Quastel, *Biochem. J.*, 1939, **33**, 1506.

[60] D. Nachmansohn and A. L. Machado, *J. Neurophysiol.*, 1943, **6**, 397; D. Nachmansohn, H. M. John, and H. Waelsch, *J. Biol. Chem.*, 1943, **150**, 485.

[61] V. A. Belitzer and E. F. Tsibakowa, *Biochimia*, 1939, **4**, 516 (cf. footnote, p. 493, ref. 63); S. Ochoa, *J. Biol. Chem.*, 1941, **138**, 751; S. P. Colowick, H. M. Kalckar, and C. F. Cori, *ibid.*, 1941, **137**, 343.

[62] S. Ochoa, *ibid.*, 1943, **149**, 577; 1944, **155**, 87.

[63] *Idem*, *ibid.*, 1943, **151**, 493.

[64] A. H. Krebs, "Advances in Enzymology," 1943, **3**, 191.

acetate oxidation generates phosphate bonds (phosphopyruvic acid) to an unknown extent.[65] The remaining dehydrogenation, of *iso*citrate to α-ketoglutarate, has not been studied in this respect. Hence, as yet only about half of the 15 ester bonds established in the oxidation of 1 mol. of pyruvate can be accounted for experimentally, and 5 such bonds are as many as could be reasonably expected on the basis of known mechanisms. It follows that there must be yet unexplored mechanisms which enable the enzyme equipment of the cell to tap the large energy range (up to 1·2 v.) between the potential levels of oxygen and of the metabolites, and turn as much as 60% of it into phosphate bond energy. F. D.

2. The Intermediary Metabolism of Tryptophan.

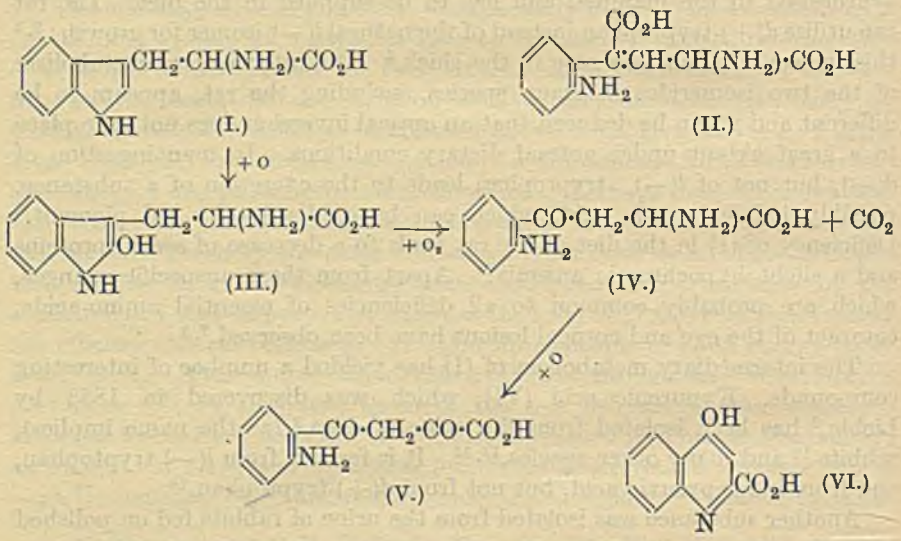
The metabolic importance of tryptophan (I) was realised soon after its discovery by F. G. Hopkins and S. W. Cole [1] in 1901. It cannot be synthesised in the mammal and has to be supplied in the diet. The rat can utilise *d*(+)-tryptophan instead of the natural *l*(−)-isomer for growth ; [2,3] this, however, is not the case in the chick.[4] The intermediary metabolism of the two isomerides in many species, including the rat, appears to be different and it can be deduced that an optical inversion does not take place to a great extent under normal dietary conditions. In man ingestion of *d*(+), but not of *l*(−), -tryptophan leads to the excretion of a substance, possibly indole-3-acetic acid, which can be oxidised to a red pigment.[5] Deficiency of (I) in the diet of the rat leads to a decrease of serum proteins and a slight hypochromic anæmia.[6] Apart from these unspecific changes, which are probably common to all deficiencies of essential amino-acids, cataract of the eye and corneal lesions have been observed.[7,8]

The intermediary metabolism of (I) has yielded a number of interesting compounds. Kynurenic acid (VI), which was discovered in 1853 by Liebig,[9] has been isolated from the urine of dogs [9] (as the name implies), rabbits [10] and many other species.[11,12] It is formed from *l*(−)-tryptophan, and from indolepyruvic acid, but not from *d*(+)-tryptophan.[13]

Another substance was isolated from the urine of rabbits fed on polished

[65] H. M. Kalckar, *J. Biol. Chem.*, 1943, **148**, 127.
[1] *J. Physiol.*, 1901, **27**, 418.
[2] C. P. Berg, *J. Biol. Chem.*, 1934, **104**, 373.
[3] V. du Vigneaud, R. R. Sealock, and L. van Etten, *ibid.*, 1932, **98**, 565.
[4] G. R. Grau and H. J. Almquist, *J. Nutrit.*, 1944, **28**, 263.
[5] A. A. Albanese and J. E. Frankston, *J. Biol. Chem.*, 1944, **155**, 101.
[6] *Idem*, *ibid.*, 1943, **148**, 299.
[7] J. R. Totter and P. L. Day, *J. Nutrit.*, 1942, **24**, 159.
[8] A. A. Albanese and W. H. Buschke, *Science*, 1942, **95**, 584.
[9] *Annalen*, **86**, 125.
[10] A. Ellinger, *Z. physiol. Chem.*, 1904, **43**, 325.
[11] W. G. Gordon, R. E. Kaufmann, and R. W. Jackson, *J. Biol. Chem.*, 1936, **113**, 125.
[12] R. W. Jackson, *ibid.*, 1939, **131**, 469.
[13] R. Borchers, C. P. Berg, and N. E. Whitman, *ibid.*, 1942, **145**, 657.

rice and supplied with excess of (I).[14] It was assigned by its Japanese discoverers the name kynurenine and the structure (II). It was shown recently that structure (II) is incorrect and that kynurenine is represented by (IV).[15] (IV) was synthesised, though in poor yield, by condensation of *o*-nitrophenacyl bromide with ethyl sodiophthalimidomalonate, followed by acid hydrolysis and reduction. The synthetic material had chemical and optical properties identical with those of the natural product; identification is, however, not quite complete, since the synthetic material has not yet been resolved. The chain of reactions leading from tryptophan (I) to kynurenic acid (VI) now becomes clear. (I) is presumably oxidised to α-hydroxytryptophan (III), a substance so far only found in phalloidin, a toxic peptide, obtained from *Aminata phalloides*;[16] (III) is then further oxidised to (IV) with loss of carbon dioxide. Under normal dietary conditions this amino-acid is further broken down, probably through the αγ-diketo-acid (V), which rearranges itself to the quinoline derivative (VI).



Still another substance derived from (I) has been isolated from the urine of rats fed on fibrin.[17] The new substance, which was called xanthurenic acid because of its yellow colour, was shown to be 4 : 8-dihydroxyquinoline-2-carboxylic acid (IX). Being an 8-hydroxyquinoline derivative, it forms complexes with metals and the intense green colour given with ferrous salts is used for its estimation. (IX) is excreted by rats,[17] rabbits,[17] and swine,[18]

[14] Y. Kotake and J. Iwao, *Z. physiol. Chem.*, 1931, **195**, 139.

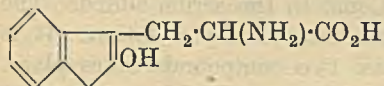
[15] A. Butenandt, W. Weidel, and W. von Derjugin, *Naturwiss.*, 1942, **30**, 51; *Z. physiol. Chem.*, 1943, **279**, 27.

[16] H. Wieland and B. Witkop, *Annalen*, 1940, **543**, 171.

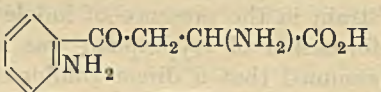
[17] L. Musajo, *Atti R. Accad. Lincei*, 1935, **21**, 368; *Gazzetta*, 1937, **67**, 165, 171, 182.

[18] G. E. Cartwright, M. M. Wintrobe, P. Jones, M. Lauritsen, and S. Humphreys, *Bull. Johns Hopkins Hosp.*, 1944, **75**, 35.

but not by dogs.[17, 19] *d*(+)-Tryptophan, indolepyruvic acid and kynurenic acid do not give rise to excretion of (IX). L. Musajo and M. Minchilli [20] claim that kynurenine does not form (IX), but Reid *et al.*[19] state that it does. The immediate precursor of (IX) may therefore be either a dihydroxytryptophan (VII) or the hydroxykynurenine (VIII).

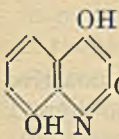


(VII.)



(VIII.)

Xanthurenic acid and kynurenine are not excreted by animals reared on normal diets, but the exact dietary deficiency necessary to produce excretion of these substances was not known until Lepkovsky *et al.*[19] showed that the green pigment found in the urines of pyridoxine deficient rats was the iron complex of (IX). Later work [18, 19] established the following facts: Xanthurenic acid is only found in pyridoxine-deficient animals fed on diets containing *l*(—)-tryptophan and the amount excreted is proportional to the tryptophan intake. Addition of pyridoxine to or omission of tryptophan from the diet leads to the disappearance of (IX) from the urine. Similarly, kynurenine excretion both in rats and in swine [18, 19] depends on pyridoxine deficiency. It seems fairly certain that kynurenine at least is a normal intermediary product of tryptophan metabolism and it appears that in pyridoxine deficiency its further breakdown is impossible. It is likely that pyridoxine is the prosthetic group of an enzyme responsible for the further oxidation of kynurenine and (IV) is excreted unchanged in its absence. (IX) may possibly be a pathological product. The fact, however, that animals on normal diets can metabolise (IX) may indicate that the formation of this 8-hydroxyquinoline derivative is an additional normal pathway of tryptophan metabolism, at least in certain species.



(IX.)

Kynurenine has recently acquired considerable interest in another direction. E. L. Tatum [21] had found that a hormone which stimulates the formation of a brown pigment in the eyes of *Drosophila* and other insects was related to tryptophan. The formation of this hormone is controlled by a specific gene and can be replaced by a substance formed by bacteria from tryptophan. This hormone was isolated by Butenandt and co-workers [15] and identified as kynurenine. E. L. Tatum and A. J. Haagen-Smit [22] showed in 1941 that their crystalline product was a complex of sucrose and kynurenine. The gene is apparently responsible for the ability of the organism to convert tryptophan into kynurenine.

[19] S. Lepkovsky, E. Roboz, and A. J. Haagen-Smit, *J. Biol. Chem.*, 1943, **149**, 195; D. F. Reid, S. Lepkovsky, D. Bonner, and E. L. Tatum, *ibid.*, 1944, **155**, 299.

[20] *Gazzetta*, 1940, **70**, 307.

[21] *Proc. Nat. Acad. Sci.*, 1939, **25**, 486; E. L. Tatum and G. W. Beadle, *Science*, 1940, **91**, 458.

[22] *J. Biol. Chem.*, 1941, **140**, 575.

Another interesting observation has recently been reported by E. L. Tatum and D. Bonner.[23] These authors found that by *X*-ray treatment a mutant of *Neurospora* can be produced which requires tryptophan for growth. This amino-acid can be replaced by a combination of indole and *l*(—)-serine, but not by any other possible intermediates. The growth of this deficient strain in the presence of indole is proportional to the serine added. The formation of tryptophan was actually demonstrated by isolation. It is assumed that a direct combination of these two compounds takes place. It is also suggested that the decomposition of tryptophan brought about by *E. coli* [24] which leads directly to indole may be a reversal of the reaction found in *Neurospora*. A. N.

3. Hormones.

The Thyroid Gland.

Thyroid Gland and Iodine Metabolism.—The application of radioactive isotopes of iodine to the study of the production of thyroxine by the thyroid gland has been very fruitful.

The investigations may be divided into two main classes: (*a*) those involving the administration of radio-iodine to the intact animal, followed by removal of the thyroid gland and other tissues for examination some hours or days later; and (*b*) those in which the uptake of radio-iodine by respiring slices of isolated thyroid tissue is examined.

(a) *Studies on the intact animal.* That iodine is preferentially retained by thyroid tissue has been amply confirmed in experiments in which radio-iodine, administered orally or parenterally, has been found in greater concentration in the thyroid gland than in any other tissue of the body within a few hours of administration.[1, 2, 3, 4] I. Perlman, I. L. Chaikoff, and M. E. Morton [3] distinguish between " tracer " doses of radio-iodine, which contain too little iodine for detection by ordinary chemical means, and what may be termed " physiological " doses of radio-iodine, measured in mg., in which a minute amount of radio-active material is mixed with ordinary potassium iodide as a carrier. When a relatively large dose (*e.g.*, 2·5 mg./kg. of body weight) of potassium iodide containing some radio-iodine is administered to a rat, 50—60% may be excreted in the urine and fæces within the next 24 hours, and only about 1% may be found in the thyroid gland. Nevertheless the thyroid gland collects, per g. of tissue, over a hundred times as much iodine as other tissues in the body and retains it longer, one-half still being present at the end of 24 hours.[3] When such large doses are administered, the thyroid tissue may become saturated with iodine and then lose its capacity to fix this element selectively, though this power may be regained within a

[23] *Proc. Nat. Acad. Sci.*, 1944, **30**, 30. [24] D. D. Woods, *Biochem. J.*, 1935, **29**, 640.

[1] S. Hertz, A. Roberts, and R. D. Evans, *Proc. Soc. Exp. Biol. Med.*, 1938, **38**, 510.

[2] (*a*) J. G. Hamilton and H. M. Soley, *Amer. J. Physiol.*, 1939, **127**, 557; (*b*) *idem*, *ibid.*, 1940, **131**, 135.

[3] *J. Biol. Chem.*, 1941, **139**, 433.

[4] C. P. Leblond and P. Sue, *Amer. J. Physiol.*, 1941, **134**, 549.

few days.[4] Radio-iodine in a non-ionic form (iodate or di-iodotyrosine) is not fixed by the thyroid tissue.[4]

The administration of a tracer dose of iodine to an animal labels its circulating iodine without significantly increasing the total amount in the blood and tissues. Such tracer doses are rapidly taken up by rat thyroid tissue, 15—20% being retained therein within 2 hours, and a maximum of 65% 30—40 hours after administration.[3] Thereafter the amount in the thyroid slowly diminishes.[3] The fact that a tissue constituting only about 0·01% of the body weight and containing approximately 20% of all the iodine in the body [5] takes up as much as 65% of the tracer dose of this element in a relatively short time suggests that the turnover of iodine by the thyroid gland is rapid, and that circulating iodine can be removed by thyroid tissue more rapidly than it can come into equilibrium with tissues other than that of the thyroid.[3] The specific activity of the thyroid gland in this respect is strikingly emphasised.

When tracer doses of iodine are given to a sheep, 2—13% is found in the thyroid gland 4 hours later. Of this about 9% is in the inorganic form, 85% as 3 : 5-di-iodotyrosine, and 6% as thyroxine. Forty-eight hours after administration 30—40% of the tracer dose is found in the gland, about 13% of this being in the inorganic form, 78% as di-iodotyrosine and 9% as thyroxine. Thus at the end of 48 hours 3—4% of the dose of administered iodine is found to be in the form of thyroxine.[6] These findings, which have been adequately confirmed, are compatible with a rapid formation of di-iodotyrosine, followed by a slower conversion of the latter into thyroxine.[6, 7] Hypophysectomy depresses the thyroid's ability to collect radio-iodine and to form di-iodotyrosine and thyroxine, but the administration of pituitary thyrotropin or exposure to cold both enhance these effects.[4, 8] In children with a myxœdema not associated with goitre the thyroid collects less administered radio-iodine than does the normal gland, whereas the thyroid of a child with a goitrous myxœdema collects more than normal.[9] In Graves' disease the hyperactive thyroid gland fixes as much as 80% of a relatively large (2 mg.) dose of administered radio-iodine,[10] and, according to C. P. Leblond,[11] the hyperplastic thyroid of iodine deficiency is also able to collect administered radio-iodine more rapidly than can the normal gland. It seems probable that increased efficiency in the collection of iodine is associated with pituitary

[5] W. T. Salter, *Physiol. Rev.*, 1940, **20**, 345.

[6] I. Perlman, M. E. Morton, and I. L. Chaikoff, *J. Biol. Chem.*, 1941, **139**, 449.

[7] (*a*) W. Mann, C. P. Leblond, and S. L. Warren, *ibid.*, 1942, **142**, 905; (*b*) A. Lein, *Endocrinology*, 1943, **32**, 429.

[8] (*a*) M. E. Morton, I. Perlman, E. Anderson, and I. L. Chaikoff, *ibid.*, 1942, **30**, 495; (*b*) M. E. Morton, I. Perlman, and I. L. Chaikoff, *J. Biol. Chem.*, 1941, **140**, 603; (*c*) C. P. Leblond, *Anat. Rec.*, 1944, **88**, 285; (*d*) C. P. Leblond, J. Gross, W. Peacock and R. D. Evans, *Amer. J. Physiol.*, 1944, **140**, 671.

[9] J. G. Hamilton, M. H. Soley, and K. B. Eichorn, *Amer. J. Dis. Child.*, 1943, **66**, 495.

[10] S. Hertz, A. Roberts, and W. T. Salter, *J. Clin. Invest.*, 1942, **21**, 25.

[11] *Rev. Canadian Biol.*, 1942, **1**, 402.

stimulation of thyroid activity, a phenomenon not observed in myxœdema resulting from pituitary deficiency.

The therapeutic value of radio-iodine retained by the thyroid in Graves' disease [12] and by metastases of thyroid carcinoma [13] is apparently disappointing, but radio-iodine is of proven value for the assessment of the completeness of thyroidectomy [14] and in examination of the functional activity of the developing thyroid gland.[15] In experiments of this type functional thyroid tissue can be detected radioautographically, *i.e.*, by its power to record its presence on a suitable photographic plate some hours after the administration to the animal of a tracer dose of radio-iodine.

(b) *Studies on slices of thyroid tissue.* When small amounts of radio-iodine in the form of potassium iodide are added to bicarbonate–Ringer solution in which are suspended surviving slices of thyroid gland, 70% of the added radio-iodine is present as di-iodotyrosine 3 hours later, and 12% as thyroxine,[16] though the addition of excess of inorganic iodide (non-radioactive) to the medium inhibits the formation of both di-iodotyrosine and thyroxine from added radio-iodine.[17] Thyroid gland which has been minced is much less effective, and a smooth suspension of finely divided tissue is almost completely inactive.[16] These results show clearly that the process of conversion of the added radio-iodine into the organic form in which it is found depends on the integrity of cell function, and is not merely the result of a chemical interchange of radio-iodine. The process is inhibited by the exclusion of air, and by addition to the medium of small amounts of cyanide, sulphide, azide and carbon monoxide, all of which inhibit the cytochrome–cytochrome oxidase system.[18] But 10^{-3}M-azide completely inhibits the formation of di-iodotyrosine and thyroxine by the slice while permitting the collection and retention in the inorganic form of 60% of the radio-iodine of the medium.[18] This and other similar evidence suggests that the thyroid mechanism for the collection of inorganic iodine can be differentiated from that responsible for the conversion of inorganic iodine into the organic form.

Non-thyroidal Production of Thyroid-active Substances.—The belief that thyroxine-like substances may be formed from administered iodine in the tissue of an animal lacking a thyroid gland [19] has been confirmed by the demonstration that both di-iodotyrosine and thyroxine are produced from

[12] (*a*) S. Hertz and A. Roberts, *J. Clin. Invest.*, 1942, **21**, 624; (*b*) J. G. Hamilton and J. H. Lawrence, *ibid.*, p. 624.

[13] A. S. Keston, R. P. Ball, V. K. Franz, and W. W. Palmer, *Science*, 1942, **95**, 362.

[14] W. O. Reinhardt, *Proc. Soc. Exp. Biol. Med.*, 1942, **50**, 81.

[15] (*a*) A. Gorbman and H. M. Evans, *ibid.*, 1941, **47**, 103; (*b*) *idem*, *Endocrinology*, 1943, **32**, 113.

[16] (*a*) M. E. Morton and I. L. Chaikoff, *J. Biol. Chem.*, 1942, **144**, 565; (*b*) *idem*, *ibid.*, 1943, **147**, 1.

[17] M. E. Morton, I. L. Chaikoff, and S. Rosenfeld, *ibid.*, 1944, **154**, 381.

[18] (*a*) H. Schachner, A. L. Franklin, and I. L. Chaikoff, *ibid.*, 1943, **151**, 191; (*b*) *idem*, *Endocrinology*, 1944, **34**, 159.

[19] (*a*) A. Chapman, *ibid.*, 1941, **29**, 686; (*b*) A. Chapman, G. M. Higgins, and F. C. Mann, *J. Endocrinol.*, 1944, **3**, 392; (*c*) I. Perlman, M. E. Morton, and I. L. Chaikoff, *Endocrinology*, 1942, **30**, 487.

radio-iodine by the fully thyroidectomised rat.[20] The minute amounts of these substances containing radio-active iodine were identified by their consistent behaviour when relatively large amounts of non-radioactive authentic substances were added as carriers during the processes of fractionation.[20] These results are of particular interest in view of the discovery, by W. Ludwig and P. von Mutzenbecher (1939),[21] that preparations of iodinated casein containing 6—8% of organically bound iodine, together with certain other iodinated proteins, possess the physiological activity of thyroid protein and yield, on alkaline hydrolysis, mono-iodotyrosine (cf. [22]), di-iodotyrosine, and pure thyroxine (100—200 mg./100 g. of iodocasein).[21] The correctness of these findings has been completely confirmed.[23] The physiologically active iodinated proteins were prepared by the addition of a limited amount of iodine to a solution of the protein in dilute sodium bicarbonate, followed by incubation at 37° for some hours. For maximal thyroid activity two atoms of iodine should be taken up for each molecule of tyrosine in the protein (Turner *et al.*[23]). P. von Mutzenbecher (1939) also showed that incubation at 37° of 3 : 5-di-iodotyrosine in alkaline solution (pH 8—9) for 1—2 weeks resulted in the formation of thyroxine in gross yield of about 0·25%,[24] and this finding, too, was amply confirmed.[25, 26, 27, 28] Von Mutzenbecher also observed that casein which had been iodinated in the cold in ammoniacal solution exhibited little or no biological activity, but that the development of biological activity resulted from incubation of this iodinated protein in alkaline solution for some days.[24] Finding that the formation of thyroxine from di-iodotyrosine by alkaline incubation was accompanied by a fall of pH (*e.g.*, from 8·8 to 8·4) and the formation of iodide, and further that the addition of sodium sulphite inhibited the formation of thyroxine whereas the addition of sodium thiosulphate did not, von Mutzenbecher suggested that the oxidation of the di-iodotyrosine to thyroxine might be associated with the splitting of iodine from di-iodotyrosine in the form of hypoiodite,[24] a suggestion that received some independent support.[26] On the other hand, the reaction, which is inhibited by the presence of potassium ferricyanide and of 3 : 5-di-iodo-4-hydroxybenzoic acid, requires the presence of air,[27] and C. R. Harington and R. V. Pitt Rivers [28b] find that it is inhibited

[20] M. E. Morton, I. L. Chaikoff, W. O. Reinhardt, and E. Anderson, *J. Biol. Chem.*, 1943, **147**, 757.

[21] *Z. physiol. Chem.*, 1939, **258**, 195.

[22] C. R. Harington and R. V. Pitt Rivers, *Biochem. J.*, 1944, **38**, 320.

[23] (*a*) *Idem*, *Nature*, 1939, **144**, 205; (*b*) E. P. Reineke, *J. Dairy Sci.*, 1942, **25**, 702; (*c*) E. P. Reineke and C. W. Turner, *Univ. Missouri Agric. Exp. Stat.*, 1942, *Res. Bull. 355*, 88 pp.; (*d*) E. P. Reineke, M. B. Williamson, and C. W. Turner, *J. Biol. Chem.*, 1942, **143**, 285; (*e*) E. P. Reineke and C. W. Turner, *J. Clin. Endocrinol.*, 1943, **3**, 1; (*f*) E. P. Reineke, M. B. Williamson, and C. W. Turner, *J. Biol. Chem.*, 1943, **147**, 115.

[24] *Z. physiol. Chem.*, 1939, **261**, 253.

[25] P. Block, jun., *J. Biol. Chem.*, 1940, **135**, 51.

[26] T. B. Johnson and L. B. Tewkesbury, jun., *Proc. Nat. Acad. Sci.*, 1942, **28**, 73.

[27] A. E. Barkdoll and W. F. Ross, *J. Amer. Chem. Soc.*, 1944, **66**, 898.

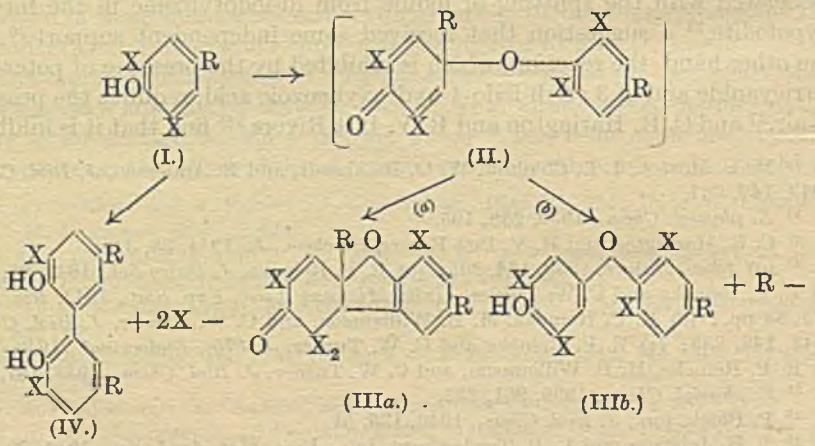
[28] (*a*) C. R. Harington, *J.*, 1944, 193; (*b*) C. R. Harington and R. V. Pitt Rivers, *Biochem. J.*, 1944, **38**, *Proc.* xxxiv.

in conditions under which the formation of hypoiodite would occur most readily. The simplest explanation might be that free iodine, which converts tyrosine into di-iodotyrosine, also brings about the oxidation of the latter to thyroxine, but von Mutzenbecher's observation that the oxidation occurs in the presence of thiosulphate is not in easy agreement with this hypothesis.

C. R. Harington [28a] has recently obtained a net yield of 3·4% of thyroxine by directly oxidising di-iodotyrosine in alkaline solution (pH 9—10) at 100° with hydrogen peroxide, the thyroxine formed being continually shaken out with butyl alcohol (a solvent into which di-iodotyrosine passes to only a slight extent from alkaline solution) in order to protect it against decomposition under the somewhat drastic conditions employed. These experiments unequivocally demonstrate that thyroxine can be formed from di-iodotyrosine by direct oxidation.

The fact that di-iodotyrosine can be so easily converted into thyroxine *in vitro* suggests the possibility that such a conversion may also easily take place in the body, but pure di-iodotyrosine possesses little or no thyroxine-like activity when administered to animals. On the other hand, J. Lerman and W. T. Salter [29] claim that the physiological activity of dried thyroid gland, which contains di-iodotyrosine, is proportional to its total iodine content and not to its variable proportion of thyroxine iodine. It seems possible, therefore, that administered peptide-linked di-iodotyrosine may be convertible into thyroxine in the body.

Mechanism of Production of Thyroxine in vitro *and* in vivo.—Harington and Barger (1927) pointed out that thyroxine might be formed *in vivo* by the coupling of two molecules of di-iodotyrosine, and this idea has recently been developed by Johnson and Tewkesbury (1942) [26] to explain the formation of thyroxine by the prolonged incubation of di-iodotyrosine in alkaline medium. These investigators recall that Pummerer *et al.*[30] oxidised *p*-cresol

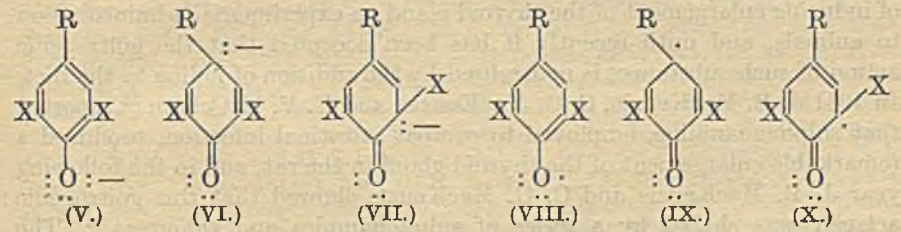


[29] *J. Pharm. Exp. Ther.*, 1934, **1**, 298.

[30] (*a*) R. Pummerer, D. Melamed, and H. Puttfarchen, *Ber.*, 1922, **55**, 3116; (*b*) R. Pummerer, H. Puttfarchen, and P. Schopflocher, *ibid.*, 1925, **58**, 1808.

with potassium ferricyanide in alkaline solution and obtained two main products: a ketotetrahydrodibenzofuran derivative (III*a*; R = Me, X = H) and 2 : 2′-dihydroxy-5 : 5′-dimethyldiphenyl (IV; R = Me, X = H). Pummerer explained the formation of (III*a*) as resulting from the rearrangement of the unstable quinonoid compound (II; R = Me, X = H), which, he suggested, was formed intermediately. Johnson and Tewkesbury pointed out that, if an analogous reaction is assumed for di-iodotyrosine, rearrangement of the intermediate quinonoid compound [II; R = CH_2·$CH(NH_2)$·CO_2H (alanyl), X = I] to a stable tetrahydrodibenzofuran derivative cannot take place, being prevented by the presence of iodine atoms *ortho* to the phenolic hydroxyl group. They suggest that the molecule may therefore stabilise itself by splitting off the alanyl side chain attached to the carbon carrying the ether oxygen, with the formation of thyroxine (III*b*; R = alanyl, X = I), and claimed to be able to identify pyruvic acid and ammonia among the products of the alkaline incubation of di-iodotyrosine, these presumably having been formed by the decomposition of the discarded alanyl side chain. Subsequently W. W. Westerfeld and C. Lowe [31] showed that the two compounds found by Pummerer *et al.* to be formed by the oxidation of *p*-cresol with ferricyanide were also obtained by oxidation of this substance with horseradish peroxidase and hydrogen peroxide.

In an interesting theoretical discussion of the mechanism of the reactions postulated by Pummerer *et al.*, Johnson and Tewkesbury, and Westerfeld and Lowe, Harington [28a] considers the implications of the assumption that *p*-cresol and di-iodotyrosine are oxidised in the form of the phenoxide ion and that the oxidation consists in the removal of an electron from the ion, followed by reaction of the free radical so formed. Harington points out that the phenoxide ion would be expected to resonate among at least three structures (V), (VI), and (VII), and that the oxidation of a *p*-substituted phenoxide ion might be assumed to consist in the removal of one electron from the oxygen atom of form (V), giving (VIII), and from the carbon atoms *para* and *ortho*, respectively, to the carbon carrying the oxygen atom of forms (VI) and (VII), giving the corresponding free radicals (IX) and (X). With *p*-cresol the interaction of (VII) and (IX) would give (II; R = Me,



X = H), which would rearrange to give (III*a*). Interaction of two molecules of (X) would give (IV). With di-iodotyrosine (R = alanyl, X = I), compound (II), formed by the interaction of (VIII) and (IX), could not stabilise as (II*a*) owing to the presence of the iodine atoms *ortho* to the phenolic

[31] *J. Biol. Chem.*, 1942, **145**, 463.

hydroxyl group, and would therefore give (III*b*) (thyroxine). For similar reasons (IV), formed from *p*-cresol, would probably not arise from di-iodotyrosine.

Chaikoff [18] suggests that the formation of both di-iodotyrosine and thyroxine by the thyroid gland is linked with aerobic oxidations in which the cytochrome–cytochrome oxidase system is involved, but E. W. Dempsey [32] believes that peroxidase is present in the thyroid follicular cells and that this enzyme may catalyse the conversion of di-iodotyrosine into thyroxine. It is possible that hydrogen peroxide is formed in living cells by the action of flavoprotein systems, and any peroxidase present might catalyse the oxidation of iodide ions to free iodine and then assist the oxidation of the di-iodotyrosine, thus formed, to thyroxine. With milk, which contains the flavoprotein system xanthine oxidase, peroxidase, and the readily iodisable protein casein, A. S. Keston [33] finds that the addition of xanthine as substrate for the xanthine oxidase system, together with a small amount of radio-iodine in the form of iodide ion, results in the rapid formation of organically bound radio-iodine. This may provide a model for further investigation of the mechanism for the organic incorporation of iodine in animal cells.

C. R. Harington [34] supports the simplest view, namely, that " the essential biochemical reaction leading to the synthesis of thyroxine may be the liberation of iodine from iodide by an oxidising enzyme system; if this were to occur conditions would be set up, namely, the presence of iodine in a faintly alkaline medium, which would not only be suitable for the iodination of tyrosine but would be analogous with those which . . . will effect the formation of thyroxine from di-iodotyrosine *in vitro*." [34] Certainly the ease with which thyroxine can be formed from tyrosine *in vitro* in the absence of enzymes but under physiological conditions not only emphasises the possibility that the formation of thyroxine from tyrosine and iodine, in the thyroid gland and elsewhere, may be a non-enzymic process but also allows consideration of the simplest hypothesis concerning the rôle of the thyroid, namely, that the primary function of this gland is the collection of circulating iodine.

Goitrogenic Substances.—For many years the existence has been realised of substances, both naturally occurring and artificial, which are capable of inducing enlargement of the thyroid gland on experimental administration to animals, and until recently it has been accepted that the goitrogenic action of such substances is neutralised by the addition of iodine to the diet. In 1941 J. B. MacKenzie, C. G. MacKenzie, and E. V. McCollum [35] reported that sulphaguanidine, employed to combat intestinal infection, produced a remarkable enlargement of the thyroid gland in the rat, and in the following year J. B. MacKenzie and C. G. MacKenzie showed that this goitrogenic activity was shared by a series of sulphonamides and thioureas.[36] The thyroid hypertrophy, which was accompanied by a fall in basal metabolic

[32] *Endocrinology*, 1944, **34**, 27. [33] *J. Biol. Chem.*, 1944, **153**, 335.
[34] *Proc. Roy. Soc.*, 1944, *B*, **132**, 223. [35] *Science*, 1941, **94**, 518.
[36] (*a*) *Federation Proc.*, 1942, **1**, 122; (*b*) *Endocrinology*, 1943, **32**, 185; (*c*) *Johns Hopkins Hosp. Bull.*, 1944, **74**, 85.

rate, was not prevented by the administration of iodine but was inhibited by the injection of thyroxine.[36] These findings were quickly confirmed,[37] and analogous results with substituted thioureas [37, 38, 39] and natural goitrogens [40] reported. The thyroid hyperplasia induced by these goitrogens was accompanied by signs of increased activity of the anterior pituitary gland, and was lacking in hypophysectomised animals.[36, 37, 40] The suggestion was then made that these substances depressed thyroid hormone production, and that the thyroid hyperplasia was secondary to increased pituitary activity evoked by the depression.[36, 37] About the same time it was observed that the prolonged administration of potassium thiocyanate to human beings could induce the appearance of thyroid goitres, associated with a fall in basal metabolic rate,[41] though the development of this type of goitre could be prevented by the administration of dietary iodine.

Acting on the assumption that thiourea interferes with the production of thyroid hormone E. B. Astwood [42] successfully treated clinical hyperthyroidism by the daily administration of thiourea and showed that 2-thiouracil also was effective. The therapeutic efficacy of this new treatment of hyperthyroidism quickly received widespread confirmation,[43] and it was shown also that the administration of thiourea or thiouracil to experimental animals duplicated the effects of thyroidectomy with respect to growth,[44] metabolism of isolated tissues,[45] organ morphology,[46] thyrotropin-induced

[37] (*a*) E. B. Astwood, J. Sullivan, A. Bissell, and R. Tyslowitz, *Endocrinology*. 1943, **32**, 210; (*b*) E. B. Astwood, *J. Pharm. Exp. Ther.*, 1943, **78**, 79; (*c*) E. W, Dempsey and E. B. Astwood, *Endocrinology*, 1944, **32**, 509.

[38] C. P. Richter and K. H. Clisby, *Arch. Path.*, 1942, **33**, 46.

[39] T. H. Kennedy, *Nature*, 1942, **150**, 233.

[40] (*a*) W. E. Greisbach and H. D. Purves, *Brit. J. Exp. Path.*, 1943, **24**, 171; (*b*) V. I. E. Whitehead, *ibid.*, p. 192.

[41] (*a*) R. W. Rawson, S. Hertz, and J. H. Means, *J. Clin. Invest.*, 1942, **21**, 624; (*b*) J. L. Kobacker, *Ohio Sta. Med. J.*, 1942, **38**, 541; (*c*) M. P. H. Foulger and E. Rose, *J. Amer. Med. Assoc.*, 1943, **122**, 1072; (*d*) R. W. Rawson, S. Hertz, and J. H. Means, *Ann. Int. Med.*, 1943, **19**, 829.

[42] *J. Amer. Med. Assoc.*, 1943, **122**, 78.

[43] (*a*) R. H. Williams and G. W. Bissell, *New England J. Med.*, 1943, **229**, 97; (*b*) H. P. Himsworth, *Lancet*, 1943, ii, 465; (*c*) R. W. Rawson, R. D. Evans, J. H. Means, W. C. Peacock, J. Lerman, and R. E. Cortell, *J. Clin. Endocrinol.*, 1944, **4**, 1; (*d*) P. B. Newcombe and E. W. Deane, *Lancet*, 1944, i, 179; (*e*) J. L. Gabrilove and M. J. Kert, *J. Amer. Med. Assoc.*, 1944, **124**, 504; (*f*) E. C. Bartels, *ibid.*, 1944, **125**, 24; (*g*) K. E. Paschkis, A. Cantarow, A. E. Rakoff, A. A. Walking, and W. J. Tourish, *J. Clin. Endocrinol.*, 1944, **4**, 179; (*h*) R. H. Williams and H. M. Chute, *New England J. Med.*, 1944, **230**, 657; (*i*) E. B. Astwood, *J. Clin. Endocrinol.*, 1944, **4**, 229; (*j*) T. H. McGavick, A. J. Gerl, M. Vogel, and D. Schwimmer, *ibid.*, p. 249; (*k*) F. L. Ritchie and B. L. Geddes, *Med. J. Aust.*, 1944, **1**, 381; (*l*) M. H. Sloan and E. Shorr, *Endocrinology*, 1944, **35**, 200; (*m*) E. B. Astwood, *ibid.*, p. 200; (*n*) H. P. Himsworth, C. A. Joll, H. Evans, G. Melton, and S. L. Simpson, *Proc. Roy. Soc. Med.*, 1944, **37**, 693; (*o*) E. M. Martin, *Canadian Med. Assoc. J.*, 1944, **51**, 39; (*p*) J. K. McGregor, *ibid.*, p. 37; (*q*) E. M. Watson and L. D. Wilcox, *ibid.*, p. 29.

[44] (*a*) A. M. Hughes, *Endocrinology*, 1944, **34**, 69; (*b*) R. H. Williams, A. R. Weinglass, G. W. Bissell, and J. B. Peters, *ibid.*, p. 317.

[45] B. J. Jandorf and R. E. Williams, *Amer. J. Physiol.*, 1944, **141**, 91.

[46] C. P. Leblond and H. E. Hoff, *Endocrinology*, 1944, **35**, 229.

metamorphosis of tadpoles,[47] development of fish,[48] pigmentation of bird plumage,[49] and insulin sensitivity.[50] Thiouracil has also been successfully employed in an evaluation of the amount of thyroxine secreted by the thyroid gland under different conditions.[37] These results all support the view that thioureas and thiouracil inhibit the formation of its hormones by the thyroid gland, but do not interfere with the action of the hormone once it has been liberated into the blood stream.

Mechanism of the Action of Thiouracil and of Other Goitrogens on the Production of Thyroid Hormone.—The daily administration of thiouracil to young rats for 8 days reduces the iodine content of the thyroid gland almost to zero, though the weight of the gland may be increased nearly threefold.[51] If the daily administration of thyroxine is now begun, with continuation of thiouracil treatment, the iodine content of the gland remains low but the follicles fill with densely staining colloid material.[32, 51] Similar results follow the removal of the pituitary gland during thiouracil administration.[51] It seems that under these conditions the secreted colloid material contains little or no thyroxine,[32, 51] and that the incorporation of iodine into this material has been inhibited by the thiouracil.

When radio-iodine is injected into rats previously made goitrous by the administration of thiouracil, the power of the thyroid gland to collect the administered iodine may be only 10—20% of normal,[52, 53, 54] and the formation of di-iodotyrosine and of thyroxine is also inhibited.[53] The capacity of thiocyanate-induced goitres to collect administered radio-iodine may be supernormal, however,[52] a finding which is significant in view of the fact that thiocyanate is an iodine-inhibited goitrogen.

A. L. Franklin, I. L. Chaikoff, and S. R. Lerner [55] found that the addition, to the medium in which surviving slices of thyroid tissue were maintained, of 10^{-3}M-thiouracil, or of a like concentration of thiourea or of potassium thiocyanate, depressed the ability of the tissue to convert added radio-iodine into di-iodotyrosine and thyroxine. This concentration of thiourea and of thiouracil had little effect on the capacity of the slices to collect iodine from the medium, although potassium thiocyanate in a similar amount significantly diminished the collection of added radio-iodine. The latter results are at variance with the data from intact animals cited above. Sulphanilamide also depresses the formation of di-iodotyrosine and thyroxine in slices.

[47] A. M. Hughes and E. B. Astwood, *Endocrinology*, 1944, **34**, 138.

[48] E. D. Goldsmith, R. F. Nigrell, A. S. Gordon, H. A. Charipper, and M. Gordon, *ibid.*, 1944, **35**, 132.

[49] M. Juhn, *ibid.*, p. 278.

[50] G. J. Martin, *Arch. Biochem.*, 1943, **3**, 61.

[51] E. B. Astwood and A. Bissell, *Endocrinology*, 1944, **34**, 282.

[52] (*a*) R. W. Rawson, J. F. Tannheimer, and W. Peacock, *ibid.*, p. 245; (*b*) R. Larson, F. R. Keating, jun., R. W. Rawson, and W. Peacock, *ibid.*, 1944, **35**, 200; (*c*) R. W. Rawson, R. E. Cortell, W. Peacock, and J. H. Means, *ibid.*, p. 201.

[53] A. L. Franklin, S. R. Lerner, and I. L. Chaikoff, *ibid.*, 1944, **34**, 265.

[54] (*a*) E. J. Baumann, N. Metzger, and D. Marine, *ibid.*, p. 44; (*b*) A. S. Keston, E. D. Goldsmith, A. S. Gordon, and H. A. Charipper, *J. Biol. Chem.*, 1944, **152**, 241.

[55] *Ibid.*, 1944, **153**, 151.

of thyroid tissue, without depressing the capacity of the slices to collect iodide from the medium.[18, 56]

As was suggested above (p. 241), the simplest hypothesis regarding the specific function of the thyroid is that this gland possesses special ability to collect iodine from the circulation. Since free iodine is presumably the iodinating agent in the formation of di-iodotyrosine from tyrosine, and since iodide ions constitute the form in which this element is collected from the blood stream, it seems probable that the first process which the collected iodide ions undergo is enzymic oxidation to free iodine. Inhibition of this process might not only inhibit the formation of di-iodotyrosine and therefore that of thyroxine but also depress the power of the gland to collect more iodide. D. Campbell, F. W. Landgrebe, and T. N. Morgan [57] recall E. A. Werner's observation [58] that free iodine can oxidise thiourea to formamidine disulphide, $NH{:}C(NH_2)\cdot S\cdot S\cdot C(NH_2){:}NH$, being itself reduced to iodide ions in the process, and suggest that this may be a mechanism whereby thiourea might interfere with the synthesis of the thyroid hormone. Another possibility is that thiourea and other similar goitrogens inhibit the action of an enzyme which catalyses the formation of iodine from iodide in the thyroid gland. Thiouracil does not poison cytochrome oxidase,[32] though cytochrome oxidase inhibitors do prevent the formation of thyroxine from inorganic iodide in surviving slices of thyroid tissue.[18] Thiouracil poisons peroxidase [32, 59] and polyphenol oxidases [60] and protects *p*-cresol against enzymic oxidation when present molecule for molecule of substrate.[60] Dempsey believes that, although peroxidase may be concerned in the formation of iodine from iodide in the thyroid gland, this enzyme also catalyses the conversion of di-iodotyrosine into thyroxine.[32] This belief is based on the observation by Dempsey and Astwood [37] that di-iodotyrosine, unlike thyroxine, does not prevent the goitrogenic action of thiouracil, the assumption being made that thiouracil must therefore inhibit the conversion of di-iodotyrosine into thyroxine. Since, however, the thyroid gland appears to be unable to utilise administered di-iodotyrosine, for the formation of thyroxine, in the absence of goitrogenic agents,[4, 43] the assumption would appear on the available evidence to be of doubtful validity.

It may be concluded that thiourea and thiouracil interfere with the formation of iodine from iodide ions, either by reducing any iodine formed back to iodide ions, or by poisoning the enzyme system catalysing the oxidation of iodide ions to iodine. Whether or not these goitrogens interfere in any other way with the formation of thyroxine in the body is as yet uncertain.

Nature of the Thyroid Hormone.—Canzanelli *et al.*[61] found that the addition of thyroglobulin, but not of thyroxine, to tissues respiring *in vitro*

[56] A. L. Franklin and I. L. Chaikoff, *J. Biol. Chem.*, 1943, **148**, 719; 1944, **152**, 295.
[57] *Lancet*, 1944, *i*, 630. [58] *J.*, 1912, **101**, 2166.
[59] J. B. Sumner and G. F. Somers, "Chemistry and Methods of Enzymes," Academic Press Inc., New York, 1943.
[60] F. Chodat and G. Duparc, *Helv. Chim. Acta*, 1944, **27**, 334.
[61] (*a*) A. Canzanelli and D. Rapport, *Endocrinology*, 1937, **21**, 779; (*b*) A. Canzanelli, R. Guild, and D. Rapport, *ibid*, 1939, **25**, 707.

increases the rate at which oxygen is taken up, and a stimulating action on tissue respiration *in vitro* has also been observed with plasma from patients with hyperthyroidism.[62] These observations suggest that thyroglobulin might be the circulating thyroid hormone, but immunological tests fail to reveal the presence of this protein in the blood stream under a variety of conditions,[63, 64] including that of hyperthyroidism.[63] Only under such an abnormal condition as thyroid trauma was thyroglobulin detected in the blood stream [63] and it seems probable that thyroglobulin as such does not normally leave the thyroid follicles. Proteolytic enzymes are present in the thyroid gland, and their activity varies under physiological conditions [65] and the hydrolysis of thyroglobulin to a less complicated thyroxine-containing molecule is probably a preliminary step in the secretion of the thyroid hormone. Harington, whose earlier results suggested that the secretion of the thyroid gland might be a thyroxine-containing peptide rather than thyroxine itself, has recently reviewed the evidence on this point [34] and concludes that there is no satisfactory reason to abandon the simplest hypothesis, namely, that thyroxine itself is the circulating hormone. As Harington and his colleagues had earlier shown,[66] the administration to rats of antisera raised against thyroxyl derivatives of horse-serum albumin and globulin confers resistance against the usual metabolism-increasing activity of administered thyroxine or thyroglobulin. That the administration of these antisera was without effect on the metabolic rate of the treated rats, though such treatment prevented the normal action of administered thyroxine and thyroglobulin, was explicable on the basis of the great power of the normal thyroid gland to respond to a call for increased secretory activity.[66] Harington [34] suggests that the simplest explanation of the facts is that the circulating antibodies of the passivity immunised animal, possessing serological combining sites adapted to thyroxine, interfere with the access of the latter to its normal sites of action in the tissues, so that it is most probable that the injected thyroxine is present as such in the circulation. He points out that this simple interpretation can be avoided only by the assumption that injected thyroxine follows the devious route of synthesis into thyroglobulin, followed by release as such (which seems on other grounds to be unlikely) or as a peptide, which is the real hormone, and such a complicated process is at least unnecessary to account for the immunological phenomena observed.[34] Harington concludes that thyroxine is "the true thyroid hormone as it circulates in the body." [34] J. H. Means [67] in another recent review concludes that "the thyroid hormone travels from the thyroid to its end-organs in a form lower than the protein level, and that it acts upon its end-organ in a form of higher level than that of the amino-acids. It may

[62] W. T. Salter and F. W. Craige, *J. Clin. Invest.*, 1938, **27**, 502.
[63] J. Lerman, *ibid.*, 1940, **19**, 555.
[64] L. I. Stellar and H. G. Olken, *Endocrinology*, 1940, **27**, 614.
[65] A. J. Dziemian, *J. Cell. Comp. Physiol.*, 1943, **21**, 339.
[66] R. F. Clutton, C. R. Harington, and M. E. Yuill, *Biochem. J.*, 1938, **32**, 1119.
[67] *Ann. Int. Med.*, 1943, **19**, 567.

both travel and act in the form of a polypeptide or peptone,"[67] a conclusion also compatible with the immunological evidence provided by Harington. If one accepts as significant the observation that thyroxine fails to stimulate tissue respiration *in vitro* whereas thyroglobulin and plasma from patients with hyperthyroidism are effective under these conditions,[61, 62] the simplest explanation of all the available evidence, including the results of the immunological investigations, appears to be that thyroxine stimulates tissue respiration only when it is combined in peptide form, and that it is transported from the thyroid tissues to the gland in this form.

In the sea, the liberation of iodine from iodine ions might occur on a minute scale wherever the oxidative catalysts of respiring cells of unicellular organisms were active. Thus the tissue proteins of a primitive protozoon might, as the result of the oxidative capacity of its enzyme systems, come to contain organically bound iodine, in the form of thyroxine, with the aid of the mechanisms reviewed above. With Means [67] we may conclude that the elaboration of the thyroid hormone preceded that of the thyroid gland in the process of evolution, and that the gland developed as an organ specialised for the production and subsequent distribution of a substance which originally was produced in the tissues in general, and which, even in higher animals, can still be made in tissues other than that of the thyroid. Thyroxine, in a combined form, may therefore be a general constituent of living protoplasm, essential for the maintenance of respiration at the high level which is characteristic of the cells of the highly developed metazoon. That being so, we might regard thyroxine not as a specific internal secretion of one ductless gland, but as an essential amino-acid.

In some respects the position with respect to choline also is analogous. Choline is an essential constituent of the normal body and the body is apparently able to manufacture all but one portion of the molecule of this important substance, namely, the methyl groups. Provided that a source of exogenous methyl groups is available to the body, *e.g.*, from methionine, choline can be manufactured in sufficient amount for its particular requirements, though otherwise this substance becomes an essential food factor and qualifies for the description of vitamin. Similarly, the only portion of the thyroxine molecule that the body cannot provide from its own resources—tyrosine is not an essential amino-acid—is iodine, and once free iodine is available the manufacture of thyroxine can proceed. In higher animals the presence of the specialised thyroid gland is essential if the rate of collection of iodine and thyroxine production are to keep pace with the demand for this amino-acid, but in lower animals the tissues in general can probably produce *in situ* all they need, provided that the essential constituent is to hand. As Means points out,[67] man could live happily without a thyroid gland if his food proteins were properly iodinated, and it is true to say that to the higher animals from which the thyroid gland has been removed thyroxine, or a thyroxine-containing iodinated protein, has become an essential constituent of the diet, and might therefore be regarded as a vitamin for such an animal.

Thyroxine, or a compound containing it in peptide linkage, can be regarded as a hormone. But such a description does not preclude the possibility of regarding it, from some points of view, as an essential amino-acid, or as a vitamin or coenzyme. Once more the overriding of boundaries which were once thought to divide different departments of scientific activity may be regarded as the natural concomitant of progress and development.

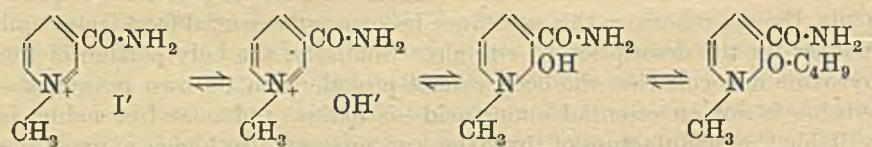
F. G. Y.

4. Nutrition.

The excretion of methylated derivatives of nicotinic acid, the relation of pyridoxine to hæmatopoiesis and iron metabolism, and the nutritional value of "folic acid" and vitamin B_c are reviewed in this section.

The Excretion of Nicotinic Acid.

In 1940 V. A. Najjar and R. W. Wood [1] described the presence in urine of a substance with a characteristic blue fluorescence, which they called F_2. Its excretion was related to the availability of nicotinic acid and increased in proportion to the nicotinic acid intake. In view of the uncertainty of the form taken by the substance in urine, the symbol F_2 is a convenient means of denoting it. F_2 is absent from the urine of pellagrins [2] and it slowly disappears from the urine of dogs fed upon a nicotinic acid-deficient diet,[3] whereas the administration of nicotinic acid or its derivatives increases its excretion.[4, 5] The chemical nature of F_2 has been elucidated and the substance has been isolated as waxy, hygroscopic, needle-shaped crystals, which in neutral or weakly alkaline conditions have a greenish-blue fluorescence, and in acidic solution a blue.[6, 7, 8] A substance identical in physical and chemical properties can be obtained by the treatment of nicotinamide methiodide with baryta. It is well known that alkalinisation of such a compound (I) will lead to the formation of a quaternary base (II), which subsequently may suffer transformation into a carbinol (III) by attachment of the hydroxyl group to one of the α-positions of the pyridine ring :



(I.) (II.) (III.) (IV.)

[1] *Proc. Soc. Exp. Biol. Med.*, 1940, **44**, 386.

[2] V. A. Najjar and L. E. Holt, *Science*, 1941, **93**, 20.

[3] V. A. Najjar, H. J. Stein, L. E. Holt, and C. V. Kabler, *J. Clin. Invest.*, 1942, **21**, 263.

[4] V. A. Najjar and L. E. Holt, *Proc. Soc. Exp. Biol. Med.*, 1941, **48**, 413.

[5] P. Ellinger and R. A. Coulson, *Biochem. J.*, 1944, **38**, 265.

[6] J. W. Huff and W. A. Perlzweig, *Science*, 1943, **97**, 538; *J. Biol. Chem.*, 1943, **150**, 395.

[7] P. Ellinger and R. A. Coulson, *Nature*, 1943, **152**, 583; *Biochem. J.*, 1943, **37**, Proc. xvii.

[8] V. A. Najjar, V. White, and D. B. N. Scott, *Bull. Johns Hopkins Hosp.*, 1944, **74**, 378.

It would appear that the substance actually isolated from urine is the carbinol. But it is uncertain [6, 7, 8] whether the fluorogenic substance in urine is the quaternary base, the ψ-base, a pyridinium salt, or a mixture of these dependent upon the conditions. The fluorescence observed *in vitro* is considered [7] to be due to a mixture of 6-hydroxy-1-methyl-1 : 6-dihydropyridine-3-carboxyamide and another carbinol with an *o*-quinonoid structure. Atmospheric oxygen and ferricyanide oxidise an alkaline solution of F_2.[8] This treatment, which leads to a deep violet fluorescence, might be expected to cause the formation of a pyridone from the base. An alternative possibility [8] is that the fluorescence is due to the formation of a carbinol ether (IV) from the ψ-base and the *iso*butanol used to extract F_2. Either suggestion would explain the slow increase in fluorescent intensity of *iso*butanol extract of F_2 from alkaline solutions.

Upon the fluorescence of the derivative of the pyridinium salt have been based methods for its estimation in urine.[9, 10, 11] The essential feature of some of them is a base exchange between the substance and permutit. The use of these methods has shown that there is a distinct individual variation and a fluctuation throughout the day in the excretion of F_2.[5] The proportionality between the amount eliminated and the intake of nicotinamide has led naturally to the development of load tests for gauging nutritional status as regards nicotinamide. Investigation of this kind of test has diminished the value of trigonelline excretion as a nutritional index, since its determination may include the pyridinium salt, which on acid or alkaline hydrolysis is converted into trigonelline.[6, 12] Nevertheless as a nutritional index the excretion of F_2 will need to be used with careful discrimination. Its dependence on the body reserves of methyl donators whose level depends upon the diet, has been indicated.[12] This view is well attested by experiments upon rats, guinea-pigs, and rabbits.[13, 14, 15] The feeding of unusual amounts of nicotinamide to rats adversely affected their growth and their livers—effects which could be remedied by choline or methionine. In guinea-pigs and rabbits no ill effects arise from the ingestion of large amounts of nicotinamide. There is a clear difference in the response of these animals to nicotinamide. The rat excretes the *N*-methylnicotinamide in the urine; the rabbit and the guinea-pig do not. Methylation of nicotinamide has been demonstrated *in vitro* with rat liver slices.[16] A consequence of a high level of nicotinamide in the diet of the rat is a depletion of its store of methyl donators, the sequelæ of which are retarded growth and fatty livers. The reverse of this, diminished stores of methionine and choline from faulty diet creating low excretion of F_2, is thus conceivable.

[9] V. A. Najjar, *Bull. Johns Hopkins Hosp.*, 1944, **74**, 392.
[10] R. A. Coulson, P. Ellinger, and M. Holden, *Biochem. J.*, 1944, **38**, 150.
[11] J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.*, 1943, **150**, 483.
[12] H. P. Sarett, *ibid.*, p. 395.
[13] P. Handler and W. J. Dann, *ibid.*, 1942, **146**, 357.
[14] P. Handler and F. Bernheimer, *J. Biol. Chem.*, 1943, **148**, 649.
[15] P. Handler, *ibid.*, 1944, **154**, 203.
[16] W. A. Perlzweig, M. L. C. Bernheim, and F. Bernheim, *ibid.*, 1943, **150** 401.

The Relation of Pyridoxine to Anæmia.

In the last decade accumulative evidence has indicated that deficiency of the B vitamins, particularly pyridoxine and nicotinic acid, may interrupt normal erythropoiesis. Mention in this review is confined to work of recent times, which attempts to define clearly the type of anæmia from blood and bone marrow investigations. The most careful work upon the effect of pyridoxine deficiency has been done on the pig and the dog.

In dogs a hypochromic anæmia would seem to be a feature of deprivation of vitamin B_6.[17,18,19,20] The anæmia does not respond to iron or copper; yet prompt improvement follows oral or intravenous administration of crystalline pyridoxine. The fact that the initial improvement is not always maintained has led to the suggestion that other factors may be involved.[19,21,22] With progressing severity of the anæmia the plasma iron rises, and rapidly falls with the administration of pyridoxine and with the initial blood regeneration.[22]

In 1938 Chick and her collaborators [23] showed that omission of the eluate fraction of a liver concentrate, a source of pyridoxine, from a synthetic diet led to a microcytic anæmia and epileptic fits in young pigs. In pigs lacking the filtrate fraction a normocytic anæmia developed. Wintrobe and his co-workers [24,25,26] found that pigs fed on a diet supplemented with vitamins A and D and all the known crystalline B vitamins except pyridoxine develop a severe microcytic anæmia which is most clearly hypochromic at its height. As the anæmia progresses, anisocytosis becomes more marked; large polychromatophilic corpuscles and cells containing blue-staining granules make an appearance. An irregular reticulocytosis may also occur. The anæmia is associated with hyperplasia of the bone marrow and an irregular reticulocytosis. The anæmia is not hæmolytic in type; no significant changes occur in the serum bilirubin or in the excretion of urobilinogen or urinary porphyrin. Fatty infiltration of the central portion of the hepatic lobules also occurs. Epileptiform convulsions are seen in the majority of B_6-deficient pigs. An outstanding feature was hæmosiderosis of the spleen, liver, and bone marrow and an increase in the serum iron, which is apparently chiefly in the ferric

[17] P. J. Fouts, O. M. Helmer, S. Lepkovsky, and J. H. Jukes, *J. Nutrition*, 1938, **16**, 197.

[18] *Idem*, *Amer. J. Med. Sci.*, 1943, **199**, 163.

[19] H. J. Borson and S. R. Mettier, *Proc. Soc. Exp. Biol. Med.*, 1940, **43**, 429.

[20] H. R. Street, G. R. Cowgill, and H. M. Zimmerman, *J. Nutrition*, 1941, **51**, 275.

[21] *Idem*, *ibid.*, p. 275.

[22] J. M. McKibbin, A. E. Schaeffer, D. V. Frost, and C. A. Elvehjem, *J. Biol. Chem.*, 1942, **142**, 77.

[23] H. Chick, J. F. Macrae, A. J. P. Martin, and C. P. Martin, *Biochem. J.*, 1938, **32**, 2207.

[24] M. M. Wintrobe, M. Samter, and H. Lisco, *Bull. Johns Hopkins Hosp.*, 1939, **64**, 399.

[25] M. M. Wintrobe, R. H. Follis, M. H. Miller, H. J. Stein, R. Alcayago, S. Hymphreys, A. Suksta, and G. E. Cartwright, *ibid.*, 1943, **72**, 1.

[26] G. E. Cartwright, M. M. Wintrobe, and S. Hymphreys, *J. Biol. Chem.*, 1944, **153**, 171.

state. Administration of pyridoxine produced a rapid regeneration of blood with a return of the red cells to normal size. This response was accompanied by a mobilisation of iron, which was indicated by the disappearance of the hæmosiderosis and a fall in the serum iron. These interesting results clearly imply a rôle for pyridoxine in iron metabolism. From the fact that in combined pyridoxine and iron deficiency no hæmosiderosis or elevated serum iron occurs despite the development of anæmia, it would appear that the disturbances in iron metabolism are due to increased absorption or decreased excretion. This is an interesting possibility, since it is contrary to the idea that the animal absorption of iron is dependent upon its needs. In many respects—the ferræmia, hæmosiderosis, hyperplastic bone marrow and neurological disturbances—the pyridoxine anæmia is similar to pernicious anæmia, although it differs in being characterised by a microcytosis and lack of response to liver extract. Nevertheless the study of the mechanism of B_6 anæmia may provide some help towards the solution of pernicious anæmia.

The possible relationship of the anæmia and kindred symptoms of vitamin B_6-deficiency with tryptophan metabolism has been referred to in Dr. Neuberger's Report (p. 237).

Folic Acid and Vitamin B_c.

During the last four years it has become evident that certain micro-organisms need for their growth one or more factors distinct from any of the known vitamins. It has also become apparent that these factors have a rôle in animal nutrition which consists, in the main, in promoting growth, countering the effect of sulphonamides, and in stimulating the formation of the cells of the blood.

In 1940 E. E. Snell and W. H. Peterson [27] described a factor of acidic nature needed by *Lactobacillus casei E*; to it they gave the name norit eluate factor. From spinach concentrates another acidic factor, named folic acid, was prepared,[28] defined as the material necessary for the growth of *Streptococcus lactis R* on a given medium. This nutrilite is abundant in green leaves and occurs in animal tissues and yeast.

Williams and his co-workers [29,30,31] have now obtained folic acid in amorphous form from spinach. It is a substance of M.W. about 400, not easily soluble in organic compounds and extremely labile. Esterification, acylation and methylation destroy its biological activity. It is also sensitive to oxidation and reduction, and is none too stable in acid or alkaline solution. From analysis it has an approximate empirical formula of $C_{15}H_{15}O_8N_5$ and absorption spectra indicate that it may contain a structural unit similar to xanthopterin.[32]

[27] *J. Bact.*, 1940, **39**, 273.
[28] H. K. Mitchell, E. E. Snell, and R. J. Williams, *J. Amer. Chem. Soc.*, 1941, **63**, 2284.
[29] *Idem, ibid.*, 1944, **66**, 267.
[30] E. H. Frieden, H. K. Mitchell, and R. J. Williams, *ibid.*, p. 269.
[31] H. K. Mitchell, and R. J. Williams, *ibid.*, p. 271.
[32] H. K. Mitchell, *ibid.*, p. 275.

From most of the work on concentrates it would appear that the norit eluate factor and folic acid are either the same substance or closely similar compounds. Concentrates of folic acid are active in stimulating the growth of yeast and other organisms, including *Lactobacillus casei*, and B. L. Hutchings, N. Bohonos, and W. H. Peterson have concluded [33] that the eluate factor was similarly of general nutritional significance for the lactic acid bacteria and the growth of *Streptococcus lactis*. From descriptions [31,34] of concentrates of folic acid it would appear that, together with folic acid, other substances of biological importance are present; these include *p*-aminobenzoic acid and xanthopterin, which are capable of counteracting the inhibitory effect of sulphonamides upon the growth of bacteria and rats. The fact that concentrates prepared from different sources stimulate the growth of *Lactobacillus casei* and *Streptococcus lactis* probably led to the interchangeable use of the terms folic acid and eluate factor. The term "folic acid" may therefore be used to indicate this group of growth stimulants.

On animals, concentrates of the eluate factor and folic acid exert effects which may be attributable to similar groups of substances. In the chick,[35] eluate factor has been found to promote growth; in the rat, folic acid.[28] Concentrates of both factors share with *p*-aminobenzoic acid the property of antagonising the noxious effects of sulphonamides, sulphaguanidine and sulphathiazole, which are poorly absorbed from the intestine. Besides producing a reduction in growth,[36] sulphonamides may cause agranulocytopenia, leucopenia, and often anæmia and other pathological conditions when they are incorporated in synthetic diets adequately supplied with vitamins.[37] Their action may be partly due to an interference with enzyme systems of the body or to suppression within the intestine of bacterial synthesis of essential factors; folic acid [38] and biotin are synthesised by intestinal bacteria. Both biotin and concentrates of the eluate factor and folic acid counteract the growth inhibition which is produced by sulphonamides.[39] Biotin and folic acid also appear to influence the utilisation of pantothenic acid by the rat. On diets abundantly supplemented with pantothenate and containing succinyl sulphathiazole, rats developed the characteristic symptoms associated with deficiency of this vitamin.[40] This change was corrected by the administration of folic acid and crystalline biotin. Although agranulocytopenia and leucopenia, produced in rats by feeding sulphonamides, respond to crystalline folic acid from different sources,[41] the effect of concentrates upon growth and blood formation may not be due solely to

[33] *J. Biol. Chem.*, 1941, **141**, 521. [34] H. K. Mitchell, *Science*, 1943, **97**, 442.
[35] B. L. Hutchings, N. Bohonos, D. M. Hegsted, C. A. Elvehjem, and W. H. Peterson, *J. Biol. Chem.*, 1940, **140**, 647.
[36] S. Black, R. S. Overman, C. A. Elvehjem, and K. P. Link, *ibid.*, 1942, **145**, 137.
[37] F. S. Daft, S. S. Ashburn, and H. H. Sebrell, *Science*, 1942, **96**, 322.
[38] H. K. Mitchell and E. R. Isbell, *Univ. Texas Pub.* No. 4327, 1942, 125.
[39] E. Nielsen and C. A. Elvehjem, *J. Biol. Chem.*, 1942, **145**, 713.
[40] L. D. Wright and A. D. Welch, *Science*, 1943, **97**, 423.
[41] F. S. Daft and W. H. Sebrell, *Pub. Health Reps. U.S.A.*, 1943, **58**, 1542.

their folic acid content. In concentrates obtained from liver, Elvehjem and his co-workers [42] claimed to have identified a growth factor, vitamin B_{11}, and a factor necessary for good feathering in chicks, vitamin B_{10}, in addition to folic acid. In several respects the properties of vitamins B_{10} and B_{11} are akin to those of folic acid.

In 1940 A. G. Hogan and E. M. Parrott [43] observed that on simplified rations chicks developed a macrocytic hypochromic anæmia which was attributed to the lack of a dietary factor, vitamin B_c, present in aqueous extracts of liver. A greater incidence of anæmia in chicks is produced by feeding sulphaguanidine.[44] Vitamin B_c is insoluble in organic solvents, more stable in alkali than in acids, adsorbable on fuller's earth and super-filtrol, and precipitable with metallic salts and phosphotungstic acid [44]—properties, in fact, similar to those of folic acid and the eluate factor. Its antianæmic action could not be reproduced by xanthopterin or by the anti-pernicious anæmia factor. Vitamin B_c has now been obtained in the crystalline form both as the free acid and as the methyl ester.[45] Incorporated in a synthetic diet amply supplemented with all the known vitamins, the crystalline substance prevented retardation in growth (both body weight and feathering) and the development of anæmia and leucopenia.[46] Given parenterally, it produced the same effects.[46] This observation has been taken to indicate that vitamin B_c produces those effects which have been claimed for folic acid and vitamins B_{10} and B_{11}. Furthermore vitamin B_c was highly active as a growth stimulant for *Lactobacillus casei E*. This led to the suggestion that vitamin B_c, the norit eluate factor and folic acid are the same substance.

The isolation of other crystalline substances has complicated rather than clarified the relationship among the microbial and the animal factors. Two crystalline compounds have been obtained; one from yeast and the other from liver.[47] Both are acids with similar absorption spectra and highly active towards *Lactobacillus casei*. There is a striking difference in their activities; towards *Lactobacillus casei* they are equally active, towards *Streptococcus lactus R* the yeast product is half as active as the liver one. Contrary to the behaviour of these crystalline acids, certain concentrates show activities greater towards *Streptococcus* than *Lactobacillus*. These facts can be harmonised by assuming the existence either of two or more substances or of different forms of one substance. In milk and in yeast folic acid may be present in a combined form, inactive to the two micro-organisms. Whole milk is more effective in inhibiting the harmful action of sulphon-

[42] G. M. Briggs, T. D. Luckey, C. A. Elvehjem, and E. B. Hart, *J. Biol. Chem.*, 1943, **148**, 163; 1944, **153**, 423.

[43] *Ibid.*, 1940, **132**, 507.

[44] B. L. O'Dell and A. G. Hogan, *ibid.*, 1943, **149**, 323.

[45] J. J. Pfiffner, S. B. Binkley, E. S. Bloom, R. A. Brown, O. D. Bird, A. D. Emmett, A. G. Hogan, and B. L. O'Dell, *Science*, 1940, **97**, 404.

[46] C. J. Campbell, R. A. Brown, and A. D. Emmett, *J. Biol. Chem.*, 1944, **152**, 483; **154**, 721.

[47] E. L. R. Stokstad, *ibid.*, 1943, **149**, 573.

amides upon rats than would be expected from its low folic acid content.[48] Yeast extracts have a high vitamin B_c activity and low microbiological activity. When submitted to enzymatic hydrolysis, they stimulate the growth of the micro-organisms. From such extracts by the same method as that used in the isolation of the antianæmic factor, a crystalline compound has been obtained which contains the same percentage amount of carbon, hydrogen, and nitrogen as vitamin B_c.[49] The individualisation of combined forms of this vitamin and the bacterial growth factors will be an important step towards an explanation of the discrepancies which have been observed in the microbial activities of different materials. It may also elucidate the relation of folic acid and vitamin B_c to vitamins B_{10} and B_{11}.

It is too early to say how important the pterins may be in animal nutrition and lack of space prohibits their inclusion here. J. R. P. O'B.

5. The Assay of Vitamins B, with Special Reference to Microbiological Methods.

The necessity of establishing nutritional requirements and levels for vitamins of group B has stimulated investigations of assay methods, and great strides have been made in recent years. These methods are of three main types: biological, microbiological, and chemical; and each has its difficulties and objections. Ideally, the three methods should be so developed that each furnishes an accurate check on the others. The development of assay methods provides an interesting example of modern collaborative work; a number of teams in this country and the United States are engaged in this manner, and some examples are quoted later.

Aneurin.—The stimulatory effect of aneurin on fermentation by living yeast has been shown to be highly specific and formed the basis of one of the first microbiological methods for the assay of the vitamin as described by A. S. Schultz, L. Atkin, and C. N. Frey.[1] These authors [2] have found that by sulphite treatment the fermentation activity of aneurin is completely (99%) destroyed, whilst interfering substances are unaffected. The authors describe a differential method, employing a new fermentometer, in which the measurement of fermentation activity is determined before and after sulphite treatment.

H. H. Bunzell [3] employs the same principle, but a different type of apparatus. Results are obtained more rapidly and are accurate for amounts of the vitamin as small as 0·01 μg. A modified Warburg technique is described by E. S. Josephson and R. S. Harris.[4]

There is in general good agreement between results by the microbiological and the chemical methods, but sometimes differences are observed when biological assays are compared. J. C. Moyer and D. K. Tressler [5] reported

[48] A. D. Welch and L. D. Wright, *Science*, 1944, **100**, 153.

[49] S. B. Binkley, O. D. Bird, E. S. Bloom, R. A. Brown, D. G. Calkins, C. J. Campbell, A. D. Emmett, and J. J. Pfiffner, *ibid.*, p. 36.

[1] *J. Amer. Chem. Soc.*, 1937, **59**, 948, 2547. [2] *Ind. Eng. Chem. Anal.*, 1942, **14**, 35.

[3] *Ibid.*, p. 279. [4] *Ibid.*, p. 755. [5] *Ibid.*, p. 788.

assays on a number of frozen vegetables in which they used fermentation and thiochrome methods successfully. R. H. Hopkins and S. Wiener [6] in a study of aneurin in the materials and process of brewing found good agreement between results by the fermentation method and the thiochrome method as described by R. G. Booth.[7] Similar satisfactory comparisons were made in a collaborative study by the Accessory Food Factors Committee of the Medical Research Council [8] in which flours and bread were assayed by several biological methods, the fermentation method, the thiochrome method and an azo method elaborated by B. S. Platt and G. E. Glock.[9]

A number of workers have reported assays using other organisms, including *Phycomyces*,[10, 11] *Phycomyces blakesleeanus*,[12–14] and *Staphylococcus aureus*.[15] These methods, however, have been criticised by C. F. Niven and K. L. Smiley [16] on various grounds. The authors claim that *Streptococcus salivarius* (Strain S20B) is more suitable. The growth response is determined turbidimetrically, and owing to the extreme sensitivity of the organism no difficulty is experienced due to incidental turbidity of added food extracts. Co-carboxylase is some 40% more active than aneurin, a fact which has not yet found explanation, and which renders enzymatic hydrolysis necessary for precise determinations in some foods.

The stability of aneurin to heat has been studied by B. W. Beadle, D. A. Greenwood, and H. R. Kraybill.[17] Stability is a function not only of the hydrogen-ion concentration of the solution, but also of the particular electrolyte system employed. Results were obtained by chemical and spectrographic examination and indicated that for a heating period of one hour at pH 5·4, there was 100% destruction at the boiling temperature in the presence of borates, as compared with 57% in unbuffered solution, 10% in the presence of acetates, and 3% where phosphates were used. R. G. Booth [18] confirms many of these findings and has extended his observations to co-carboxylase, which he finds very much less stable than aneurin at the same pH. Destruction of aneurin is not primarily an oxidation effect, although, as copper can catalyse destruction, oxidation may be involved. An everyday application of work of this type concerns the losses of the vitamin which occur in cooking. Booth found that his estimate of loss agreed reasonably well with published figures.

Riboflavin.—Although both the microbiological and the fluorimetric methods for assay of riboflavin have yielded results in reasonable accord

[6] *J. Inst. Brew.*, 1944, **41**, 124.
[7] *J. Soc. Chem. Ind.*, 1940, **59**, 181.
[8] *Biochem. J.*, 1943, **37**, 433.
[9] *Ibid.*, p. 439.
[10] M. Malm and H. Lundeen, *Svensk Kem. Tid.*, 1941, **53**, 246.
[11] J. Lehmann and H. E. Nielsen, *Acta Med. Skand.*, Suppl., 1941, **123**, 374.
[12] W. H. Schopfer and A. Jung, *Compt. rend.*, 1937, **204**, 1500.
[13] J. Bonner and J. Erickson, *Amer. J. Bot.*, 1938, **25**, 685.
[14] J. Meiklejohn, *Biochem. J.*, 1943, **37**, 349.
[15] P. M. West and P. W. Wilson, *Science*, 1938, **88**, 334.
[16] *J. Biol. Chem.*, 1943, **150**, 1.
[17] *Ibid.*, 1943, **149**, 339, 349.
[18] *Biochem. J.*, 1943, **37**, 518.

with those obtained by biological methods, much evidence has accumulated that interfering substances may be present in natural products. It is necessary that each type of product should be treated in relation to its own peculiarities and the problems arising therefrom. In the fluorimetric method, originally developed by A. Z. Hodson and L. C. Norris,[19] later modified by V. A. Najjar,[20] pigments and non-flavin fluorescent substances must either be removed or allowed for.

The original microbiological assay method of E. E. Snell and F. M. Strong[21] used *Lactobacillus casei*-ε as test organism. Henceforth this organism will be denoted by its more convenient synonym, *Lactobacillus helveticus*.

In a study of assay methods for cereals, J. S. Andrews, H. M. Boyd, and D. E. Terry[22] found that the method of extraction is of great importance if satisfactory results are to be obtained. Extraction with taka-diastase was necessary in order to eliminate the effects of undesirable impurities. In this manner agreement was obtained between results by the microbiological method and the fluorimetric method in the case of patent and whole wheat flours, but there were discrepancies in the case of other cereal products. On the other hand, M. I. Wegner, A. R. Kemmerer, and G. S. Fraps[23] found taka-diastase (and also papain) treatment unsatisfactory in microbiological work on similar products, nor could the difficulty be obviated by adding photolysed extracts to the basal medium.

J. C. Bauernfeind, A. L. Sotier, and C. S. Boruff[24] found that the effect of additional growth substances in some foodstuffs was observable in assays using *L. helveticus*, especially when the amounts of riboflavin were below the optimum. The authors described methods for countering these effects, and suggested that the interfering substances were of the nature of fatty acids. This suggestion was followed up in an important paper by F. M. Strong and L. E. Carpenter,[25] who examined the effects of added fatty acids, to which the organism was sensitive, and showed that the difficulty did in fact arise from their presence. If they are removed by suitable preliminary treatment, reliable values for riboflavin may be obtained.

Satisfactory concordance in results by the microbiological method, which was modified by E. C. Barton-Wright and R. G. Booth,[26] and the fluorimetric method, as adapted by V. A. Najjar,[20] has been achieved by these authors in the assay of many cereals and cereal products. D. W. Kent-Jones and M. Meiklejohn[27] also have obtained satisfactory results by these methods.

R. H. Hopkins and S. Wiener[6] give figures for riboflavin in brewing materials by the microbiological method, but indicate that additional investigation of the fluorimetric method is necessary owing to disturbing factors in such materials as hops.

[19] *J. Biol. Chem.*, 1939, **131**, 621.
[20] *Ibid.*, 1941, **141**, 355.
[21] *Ind. Eng. Chem. Anal.*, 1939, **11**, 346.
[22] *Ibid.*, 1942, **14**, 271.
[23] *J. Biol. Chem.*, 1942, **144**, 731.
[24] *Ind. Eng. Chem. Anal.*, 1942, **14**, 666.
[25] *Ibid.*, p. 909.
[26] *Biochem. J.*, 1943, **37**, 25.
[27] *Analyst*, 1944, **69**, 330.

Finally, a collaborative study of the riboflavin content of meals served in R.A.F. messes may be mentioned. In this instance good agreement was obtained between the biological and the microbiological methods and it is concluded by T. F. Macrae, E. C. Barton-Wright, and A. M. Copping [28] that the adult riboflavin requirement does not exceed 2 mg. per day.

Nicotinic Acid.—An excellent review on nicotinic acid is contributed by C. A. Elvehjem and L. J. Tepley.[29]

There are a large number of chemical methods and their modifications for the estimation of nicotinic acid. All depend on the reaction with cyanogen bromide, followed by colour production with an amine.[30] Probably the most extensive study has been made by E. Kodicek,[31] who later modified the procedure in collaboration with Y. L. Wang.[32] The colour-producing base employed in both methods is *p*-aminoacetophenone; other bases proposed include orthoform (orthocaine),[33] *p*-phenylenediamine dihydrochloride,[34] and procaine.[35] The last gave good results with animal products such as meat extract and meat juice; but in general it may be said that the chemical methods are unreliable for plant products.

The method of E. E. Snell and L. D. Wright [36] was modified by W. A. Krehl, F. M. Strong, and C. A. Elvehjem,[37] who employed *Lactobacillus arabinosus* 17/5 and a synthetic medium.

In a study of methods of extraction V. H. Cheldelin and R. R. Williams [38] find that many materials yield their nicotinic acid completely under digestion with taka-diastase and papain, and that similar values in the case of meats and milk are obtained whether hydrolysis is enzymatic or by acid or alkali. On the other hand, acid or alkaline extracts of cereals give higher values than those prepared by enzyme action.

Comparison of results by microbiological and chemical methods of assay has shown that higher results by microbiological assays are obtained when plant products, particularly cereals, are treated in the preliminary stage with acid. R. D. Greene, A. Black, and F. O. Howland [39] employed a method similar to that of Snell and Wright [36] for microbiological assays, and a modified cyanogen bromide method due to W. S. Jones.[40] With some products, good agreement was found between the two types of method, although the authors prefer the microbiological method where small quantities of nicotinic acid are present. J. A. Andrews, H. M. Boyd, and W. A. Gortner [41] have studied the nicotinic acid content of cereals and cereal

[28] *Biochem. J.*, 1944, **38**, 132.
[29] *Chem. Reviews*, 1943, **33**, 185.
[30] W. König, *J. pr. Chem.*, 1904, **69**, 105.
[31] *Biochem. J.*, 1940, **34**, 724.
[32] *Ibid.*, 1943, **37**, 530.
[33] R. G. Martinek, E. R. Kirch, and G. L. Webster, *J. Biol. Chem.*, 1943, **149**, 245.
[34] A. E. Teeri and S. R. Shimer, *ibid.*, 1944, **153**, 307.
[35] E. C. Barton-Wright and R. G. Booth, *Lancet*, 1944, 565.
[36] *J. Biol. Chem.*, 1941, **139**, 675.
[37] *Ind. Eng. Chem. Anal.*, 1943, **15**, 471.
[38] *Ibid.*, 1942, **14**, 671.
[39] *Ibid.*, 1943, **15**, 77.
[40] *J. Amer. Pharm. Assoc.*, Sci. Ed., 1941, **30**, 272.
[41] *Ind. Eng. Chem. Anal.*, 1942, **14**, 663.

products, and also conclude that the microbiological assay is influenced by the type of hydrolysis procedure employed.

Nevertheless, the method of Krehl, Strong, and Elvehjem [37] is proving most valuable, and has recently reached a high level of accuracy as modified by E. C. Barton-Wright,[42] who has applied it to a wide range of materials, which are extracted under pressure with N-hydrochloric acid. Fats and fatty acids do not appear to have any effect on the organism. D. W. Kent-Jones and M. Meiklejohn [27] have applied the method with success.

Pyridoxine.—Colorimetric methods for assay of pyridoxine have been proposed by M. Swaninathan [43] and by J. V. Scudi.[44] Modifications of the latter method have been suggested by O. D. Bird, J. M. Vanderbelt, and A. D. Emmett,[45] and by A. F. Bina, J. M. Thomas, and E. B. Brown.[46] The most recent reference to such methods is probably that by A. C. Bottomley.[47]

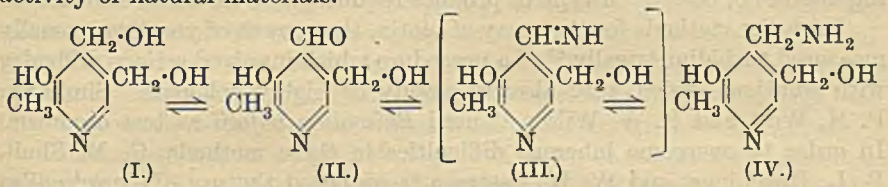
A yeast growth method originally presented by L. Atkin, A S. Schultz, and C. N. Frey [48] has been modified by these authors together with W. L. Williams.[49] The organism used is a yeast strain (No. 4228) which is characterised by a specific response to pyridoxine. Extracts of the materials for assay are prepared by acid treatment, and yeast growth is estimated turbidimetrically. Satisfactory assays on a large number of substances are reported. Bound pyridoxine is liberated also by acid treatment under pressure by L. Siegel, D. Melnick, and B. L. Osler.[50] Their results for a number of natural materials agreed well with those obtained by biological methods.

It was shown by E. E. Snell, B. M. Guirard, and R. J. Williams [51] that *Streptococcus lactis* R would grow on a medium if in addition to the usual constituents pyridoxine were present. Growth on such a medium, however, was many times as great as could be accounted for on the basis of actual content of pyridoxine. The indications were that pyridoxine is converted into a more highly active metabolite, called ψ-pyridoxine for the present, prior to utilisation by the organism, and that ψ-pyridoxine exists in natural products. The original presence or derivation of pyridoxine renders microbiological assays for pyridoxine invalid, and the case is complicated by the fact that the effect varies with different organisms; *e.g.*, very high values are obtained as indicated with *Streptococcus lactis* R, but low values are obtained with *Saccharomyces cerevisiæ* as test organism.

In a later communication, E. E. Snell [52] advances suggestions as to the nature of ψ-pyridoxine, and shows that mixtures having enhanced growth-promoting properties for *Lactobacilli* may be formed from pyridoxine by processes involving (*a*) possible amination and (*b*) partial oxidation. The latter change had also been noted by L. E. Carpenter and F. M. Strong.[53]

[42] *Biochem. J.*, 1944, **38**, 314.
[43] *Indian J. Med. Res.*, 1941, **29**, 561.
[44] *J. Biol. Chem.*, 1941, **139**, 707.
[45] *Ibid.*, 1942, **142**, 317.
[46] *Ibid.*, 1943, **148**, 111.
[47] *Biochem. J.*, 1945 (in the press).
[48] *J. Amer. Chem. Soc.*, 1939, **61**, 1931.
[49] *Ind. Eng. Chem. Anal.*, 1943, **15**, 141.
[50] *J. Biol. Chem.*, 1943, **149**, 361.
[51] *Ibid.*, 1942, **143**, 519.
[52] *Ibid.*, 1944, **154**, 313.
[53] *Arch. Biochem.*, 1944, **3**, 375.

An amine (IV) and an aldehyde (II), " pyridoxamine " and " pyridoxal " respectively, have been synthesised,[54] and there is much evidence that these compounds or their higher combinations are responsible for the ψ-pyridoxine activity of natural materials.



(I.) (Pyridoxine) (II.) (III.) (IV.)

The use of biochemical mutants in the mould *Neurospora* induced by means of ultra-violet and *X*-rays is an interesting development in microbiological methods of assay of vitamins of the B group. The production of these mutants has been described by G. W. Beadle and E. L. Tatum,[55] and they are characterised by an inability to carry out specific syntheses which can be effected by the normal unmutated strain.

An *X*-ray-induced mutant of *Neurospora sitophila*, produced by Beadle and Tatum is utilised as test organism by J. L. Stokes, A. Larsen, C. R. Woodward, and J. W. Foster [56] in a microbiological method for pyridoxine. Growth response is determined by actual dry weight of the mould, and the method is thus free from some objections which arise in turbidimetric assays. Under the conditions employed, the organism exhibits a specific response to pyridoxine, but none to ψ-pyridoxine. The results obtained are in good agreement with those obtained by biological assay.

Biotin.—The elucidation of the structure of biotin has been discussed in detail.[57] The importance of this needs no stressing, since, apart from scientific interest in the substance itself, it is a valuable tool in much modern microbiological work.

It may be some time before a chemical test for biotin of the required delicacy and specificity is forthcoming. In the meantime, the microbiological methods are being intensively studied, one of the more important problems centring on the question of free and bound biotin. Earlier methods of extraction included treatment merely with hot water,[58] but it was later found that much larger amounts of biotin were yielded by autolysis of tissues such as liver.[59] Later still,[60, 61] a combination of autolysis and acid hydrolysis was resorted to, and in 1941, after a series of tests of all types of treatment, R. C. Thompson, R. E. Eakin, and R. J. Williams [62] came to the conclusion that the best method for many types of material consists in drastic

[54] S. A. Harris, D. Heyl, K. Folkers, and E. E. Snell, *J. Biol. Chem.*, 1944, **154**, 315.
[55] *Proc. Nat. Acad. Sci.*, 1941, **27**, 499; 1942, **28**, 234.
[56] *J. Biol. Chem.*, 1943, **150**, 17.
[57] *Ann. Reports*, 1943, **40**, 172.
[58] F. Kögl and W. van Hasselt, *Z. physiol. Chem.*, 1936, **243**, 189.
[59] E. E. Snell, R. E. Eakin, and R. J. Williams, *J. Amer. Chem. Soc.*, 1940, **62**, 175.
[60] R. E. Eakin, W. A. McKinley, and R. J. Williams, *Science*, 1940, **92**, 224.
[61] *Univ. Texas Publication*, 1941, No. 4137.
[62] *Science*, 1941, **94**, 589.

acid treatment. Some destruction of the biotin occurs, but it is remarkably stable in acid solution. The problem is complicated by the fact that biotin appears to exist in different combinations which are broken down with varying degrees of ease, each type of product requiring individual treatment.

In earlier methods for the assay of biotin, the growth of yeast was usually measured turbidimetrically,[63, 64] a procedure which involved serious difficulty with solutions which were already cloudy or highly coloured. Similarly, P. M. West and P. W. Wilson [65] used *Rhizobium trifolii* as test organism. In order to overcome inherent difficulties in these methods, G. M. Shull, B. L. Hutchings, and W. H. Peterson [66] proposed the use of *Lactobacillus helveticus* as test organism, and measured the effect of added biotin by the increase in titratable acidity. An added advantage of this method lies in the fact that the same organism may be used for assay of pantothenic acid and riboflavin, thus obviating additional cultures. G. M. Shull and W. H. Peterson [67] later suggested two modifications in the assay. The eluate factor level in the yeast supplement in the basal medium is increased so that optimal growth of the organism is obtained. A procedure whereby the inoculum is independent of drop size is described.

The chemistry and biochemistry of biotin is reviewed by K. Hofmann.[68] A detailed account of methods and results of microbiological assay of all vitamins in the B group is provided by R. J. Williams and his collaborators.[69]

Pantothenic Acid.—No satisfactory chemical method of assay of pantothenic acid has as yet been devised. The earlier microbiological methods based on stimulation of yeast growth have largely given place to methods in which *L. helveticus* is used as test organism.[70–75]

The method of Pennington *et al.* employed autoclaving with or without previous autolysis under benzene in order to free pantothenic acid from test materials. Various enzymatic methods have been employed.[74, 76–78]

In more recent studies on the microbiological assay, A. L. Neal and F. M. Strong [79] have endeavoured to overcome some of the difficulties previously

[63] F. Kögl and B. Tonnis, *Z. physiol. Chem.*, 1936, **242**, 43.
[64] E. E. Snell, R. E. Eakin, and R. J. Williams, *J. Amer. Chem. Soc.*, 1940, **62**, 175.
[65] *Enzymologia*, 1940, **8**, 152. [66] *J. Biol. Chem.*, 1942, **142**, 913.
[67] *Ibid.*, 1943, **151**, 201.
[68] "Advances in Enzymology," 1943, **3**, 289. Interscience Publishers, New York.
[69] *Univ. Texas Publications*, 1941, No. 4137; 1942, No. 4237.
[70] E. E. Snell, F. M. Strong, and W. H. Peterson, *Biochem. J.*, 1937, **31**, 1789.
[71] *Idem, J. Amer. Chem. Soc.*, 1938, **60**, 2825.
[72] *Idem, J. Bact.*, 1939, **38**, 293.
[73] D. Pennington, E. E. Snell, and R. J. Williams, *J. Biol. Chem.*, 1940, **135**, 213.
[74] F. M. Strong, R. E. Feeney, and A. Earle, *Ind. Eng. Chem. Anal.*, 1941, **13**, 566.
[75] D. Pennington, E. E. Snell, H. K. Mitchell, J. R. McMahan, and R. J. Williams, *Univ. Texas Publication*, 1941, No. 4137, 14.
[76] H. A. Waisman, L. M. Henderson, J. M. McIntire, and C. A. Elvehjem, *J. Nutrition*, 1942, **23**, 239.
[77] A. H. Buskirk and R. A. Delor, *J. Biol. Chem.*, 1942, **145**, 707.
[78] E. Willerton and W. H. Cromwell, *Ind. Eng. Chem. Anal.*, 1942, **14**, 603.
[79] *Ibid.*, 1943, **15**, 654.

encountered by modifying the medium employed and improving the method of growing the inoculum. Enzymatic methods of liberating "bound" pantothenic acid were studied until satisfactory results were obtained and steps were taken to eliminate interfering fat-soluble substances.[24,25] The effect of water-soluble substances, present particularly in brans, was minimised by modifications in the basal medium. The authors claim that the modified method gives concordant results at increasing levels of dosage, and that very small amounts of the vitamin may be estimated with accuracy.

There appears to be an additional growth factor or factors for *L. helveticus* in the concentrate of rice polishings according to M. F. Clarke, M. Lechycka, and A. E. Light.[80] Notable increases in acid production were observed over and above those normally experienced with pure calcium pantothenate. The high values obtained by these workers may not, however, necessarily be due to a supplementary growth stimulator. J. L. Stokes and B. B. Martin [81] report that high acid production may be obtained merely by increasing the amounts of glucose and sodium acetate in the medium. With a view to increasing acid production and hence the titration range, A. E. Light and M. F. Clarke [82] propose a modification in the medium.

Other test organisms have been employed, among which *Streptococcus lactis*,[83] *Streptobacterium plantarum*,[84] *Proteus morganii*,[84a] and *L. arabinosus* [84b] may be mentioned.

A useful review of pantothenic acid is contributed by R. J. Williams.[85]

p-*Aminobenzoic Acid.*—Chemical methods are not greatly in evidence as yet, but are being developed. Colorimetric methods are described by E. R. Kirch and O. Bergeim [86] and by H. W. Eckert.[87]

Acetobacter suboxydans is recommended as test organism for *p*-aminobenzoic acid by M. Landy and D. M. Dicken,[88] who describe a suitable basal medium. Related or derived compounds of *p*-aminobenzoic acid have little or no biological activity, and the method has high specificity.

A mutant strain of *Neurospora crassa* of G. W. Beadle and E. L. Tatum [55] is used by R. C. Thompson, E. R. Isbell, and H. K. Mitchell.[89] Additions of graded amounts of *p*-aminobenzoic acid to a synthetic medium stimulate a specific growth response in the mould which is determined by measurement of the growth produced. The extraction of *p*-aminobenzoic acid by water and by acid hydrolysis is compared. The latter treatment involves a certain loss of the vitamin, but this loss is not significant in comparison with the enhanced yield of "bound" *p*-aminobenzoic acid. The same authors [90] have later shown that complete extraction is effected only by acid hydrolysis

[80] *J. Biol. Chem.*, 1942, **142**, 957. [81] *Ibid.*, 1943, **147**, 483. [82] *Ibid.*, p. 739.
[83] H. K. Mitchell, H. H. Weinstock, E. E. Snell, S. R. Stanbury, and R. J. Williams, *J. Amer. Chem. Soc.*, 1940, **62**, 1776.
[84] R. Kuhn and T. Wieland, *Ber.*, 1940, **73**, 962.
[84a] M. J. Pelczar and J. R. Porter, *J. Biol. Chem.*, 1941, **139**, 675.
[84b] H. R. Skeggs and L. D. Wright, *ibid.*, 1944, **156**, 21.
[85] "Advances in Enzymology," 1943, **3**, 253. Interscience Publishers, New York.
[86] *J. Biol. Chem.*, 1943, **148**, 445. [87] *Ibid.*, p. 197.
[88] *Ibid.*, 1942, **146**, 109. [89] *Ibid.*, 1943, **148**, 281. [90] *Ibid.*, 1943, **147**, 485.

under pressure. They suggest that the method of Landy and Dicken [88] responds to only a fraction of the total yielded by acid hydrolysis.

Quantitative response to *p*-aminobenzoic acid is evinced by *Clostridium acetobutylicum* Strain S9, which attains maximal growth in 24 hours on a suitable medium proposed by J. O. Lampen and W. H. Peterson.[91] These authors claim that the vitamin is rapidly destroyed by acid hydrolysis, and prefer to hydrolyse with alkali under pressure. This method of extraction is also favoured by J. C. Lewis,[92] who uses *L. arabinosus* as test organism.

Reference should not be omitted to the synthetic medium of M. Landy and D. M. Dicken [93] for use with *L. helveticus* and applicable to assay of each member of the group. Whilst this ideal has not perhaps been realised, the medium or modifications of it have proved useful to many workers.

The family of B vitamins is ever-increasing and it is too early to discuss assay methods for new members. It may be mentioned, however, that methods for " folic acid " are available.[69] F. W. N.

6. Actions of Chemotherapeutic Agents and Related Compounds.

Chemotherapy concerns interactions of drug, parasite and host, but the majority of investigations of chemotherapeutic agents during the period reviewed have been of their effects upon bacteria. The present account is mainly limited to such effects and is arranged according to their type. Factors involved in the comparison of *in vivo* and *in vitro* actions of drugs have been examined,[1] and their relations to other interactions in the complete chemotherapeutic system have been reviewed elsewhere.[2]

I. *Biological Effects.*

(a) *Morphological.*—Abnormal size or shape in bacterial cells is induced by many agents;[3, 4] sometimes but not always [5] by sulphanilamide, and frequently by compounds without known chemotherapeutic action.[5] Their occurrence in response to changes in media has been ascribed to independent effects of the change upon chemical factors conditioning cell elongation and division.[5, 6]

(b) *Upon Growth.*—Sulphonamides increase the mean generation time during the logarithmic phase; and the length of the lag phase, of *Bact. lactis ærogenes*;[7] pantoyltaurine, in concentrations active *in vivo* against *Strepto-*

[91] *J. Biol. Chem.*, 1944, **153**, 193. [92] *Ibid.*, 1942, **146**, 441.
[93] *J. Lab. Clin. Med.*, 1942, **27**, 1086.

[1] Symposia, *Trans. Faraday Soc.*, 1943, **39**, 319; *Ann. N.Y. Acad. Sci.*, 1943, **44**, 445.
[2] H. McIlwain, *Biol. Rev.*, 1944, **19**, 135.
[3] *E.g.*, J. W. Foster and H. B. Woodruff, *Arch. Biochem.*, 1943, **3**, 241.
[4] G. H. Spray and R. M. Lodge, *Trans. Faraday Soc.*, 1943, **39**, 424.
[5] C. N. Hinshelwood and R. M. Lodge, *Proc. Roy. Soc.*, 1944, *B*, **132**, 47.
[6] R. M. Lodge and C. N. Hinshelwood, *Trans. Faraday Soc.*, 1943, **39**, 420.
[7] D. S. Davies and C. N. Hinshelwood, *ibid.*, p. 431.

coccus hæmolyticus, has effects upon that organism which are similar and which, like the *in vivo* activity, are annulled by pantothenate.[8] The effects of many other metabolite-analogues upon overall growth have been reported. Pyrithiamine, in which a pyridine ring replaces the thiazole ring of aneurin, inhibits several bacteria [9, 10] and has greatest effects on those most exacting in their requirements for aneurin,[9] which antagonises its action. Inhibition by benziminazole is counteracted by some aminopurines.[11] Dethiobiotin,[12] biotin sulphone,[13] and an analogous imidazolidone derivative [14] competitively inhibit certain organisms but to others may be indifferent or in some cases act as source of biotin; or they may make available to bacteria, biotin which is inactivated by avidin. 6 : 7-Dichloro-9-ribityl*iso*alloxazine [15] and phenazine analogues of riboflavine [16] inhibit bacterial growth and this may be restored by riboflavine. New analogues of *p*-aminobenzoate [17] and pantothenate,[18] some of which are antibacterial, have been reported. Inhibitory substances designed in this way can act upon strains of organisms resistant to the agent used as model,[19] though cross-resistance can be developed to agents apparently different in type.[20] Orthanilamide does not inhibit an organism to which anthranilic acid is a growth-factor.[21]

Considering existing chemotherapeuticals, the inhibition of growth of *Escherichia coli* caused by atebrin [22] and of a lactobacillus and streptococcus caused by diamidines [23] are antagonised by spermidine and polyamines. The interaction of *p*-aminobenzoate and sulphonamides has been investigated under various conditions of aeration [24] and temperature.[25] The latter factor influences also the mutual interaction of *p*-aminobenzoate, sulphonamides and urea; [26] joint action of the last two can be additive, as urea is sometimes bacteriostatic.[27] Effects of sulphonamides on certain micro-

[8] H. McIlwain, *Biochem. J.*, 1944, **38**, 97.
[9] D. W. Woolley and A. G. C. White, *J. Exp. Med.*, 1943, **78**, 489.
[10] O. Wyss, *J. Bact.*, 1943, **46**, 483.
[11] D. W. Woolley, *J. Biol. Chem.*, 1944, **152**, 225.
[12] K. Dittmer, D. B. Melville, and V. du Vigneaud, *Science*, 1944, **99**, 203; V. G. Lilly and L. H. Leonian, *ibid.*, p. 205.
[13] K. Dittmer, V. du Vigneaud, P. Gyorgi, and C. S. Rose, *Arch. Biochem.*, 1944, **4**, 229.
[14] K. Dittmer and V. du Vigneaud, *Science*, 1944, **100**, 129.
[15] R. Kuhn, F. Weygand, and E. F. Möller, *Ber.*, 1943, **76**, 1044.
[16] D. W. Woolley, *J. Biol. Chem.*, 1944, **154**, 31.
[17] O. H. Johnson, D. E. Green, and R. Pauli, *ibid.*, 1944, **153**, 37.
[18] J. Barnett, *J.*, 1944, 5; J. Barnett, D. J. Dupré, B. J. Holloway, and F. A. Robinson, *ibid.*, p. 94.
[19] H. McIlwain, *Brit. J. Exp. Path.*, 1943, **24**, 203.
[20] J. McIntosh and F. R. Selbie, *ibid.*, p. 246.
[21] E. E. Snell, *Arch. Biochem.*, 1943, **2**, 389.
[22] M. Silverman and E. A. Evans, jun., *J. Biol. Chem.*, 1943, **150**, 265; 1944, **154**, 521.
[23] E. E. Snell, *ibid.*, 1943, **152**, 475.
[24] J. W. McLeod, A. Mayr-Harting, and N. Walker, *J. Path. Bact.*, 1944, **56**, 377.
[25] S. W. Lee and E. J. Foley, *Proc. Soc. Exp. Biol. Med.*, 1943, **53**, 243.
[26] S. W. Lee, J. A. Epstein, and E. J. Foley, *ibid.*, p. 245.
[27] W. M. M. Kirby, *ibid.*, p. 109.

organisms differ from the compounds' normal antibacterial effects in not being antagonised by *p*-aminobenzoate.[28] Lack of such antagonism is a valuable feature in the homosulphonamides, of which new members are active *in vivo*.[29]

(c) *Upon Viability.*—An outstanding finding of the period under review is of the unusual action of penicillin. At concentrations approximating to those attained during therapy, penicillin has little effect upon the viability of staphylococci,[30] hæmolytic streptococci[31] and meningococci[32] under conditions which do not permit growth of the organisms; *e.g.*, in salt solutions or in very dilute broth, in rich media in the cold or in rich media when growth is inhibited by sulphonamides[33] or by boric acid.[30] Under conditions otherwise permitting growth, an extremely small concentration of penicillin is bacteriostatic, 0·0009 unit/ml. (*c.* 0·0005 μg./ml.) having an effect comparable with that of 100 μg./ml. of sulphadiazine; concentrations comparable with those used therapeutically (*e.g.*, of 1/24 unit/ml.) are, however, bactericidal. Factors which normally increase the rate of growth of streptococci, in the presence of penicillin increase their rate of death. A proportion of organisms in staphylococcal cultures is not susceptible to being killed by penicillin; such " persistent " organisms are considered to be in a particular cultural phase. The proportions of organisms of a culture which are persistent can be altered by manipulation of the culture;[30] they increase on chilling. Recommendations in the clinical use of penicillin have been made on the basis of the new findings.[30] Varying susceptibility of bacteria at different phases of the culture-cycle has frequently been observed[34] and a further example has appeared recently in the greater sensitivity to acriflavine of *B. salmonicida* while it is in its logarithmic phase.[35]

Of agents already known to be bactericidal, the relations between concentration and action[36] and time of exposure and action[37] of phenol have been further studied. The significance of rates of death has been discussed.[38] Surface-active cations such as benzylalkylammonium chlorides are bactericidal, but their toxic action upon bacteria can be prevented, and when in progress halted, by anions of large molecular weight such as sodium dodecyl sulphate.[39] This shows two phases in the action of the cation: a pre-

[28] J. T. Tamura, *J. Bact.*, 1944, **47**, 529; F. Hawking, *Brit. J. Exp. Path.*, 1944, **25**, 63.

[29] D. M. Hamre, H. A. Walker, W. B. Dunham, H. B. van Dyke, and G. Rake, *Proc. Soc. Exp. Biol., N.Y.*, 1944, **55**, 170; D. G. Evans, A. T. Fuller, and J. Walker, *Lancet*, 1944, **247**, 523.

[30] J. W. Bigger, *ibid.*, p. 497.

[31] G. L. Hobby and M. H. Dawson, *Proc. Soc. Exp. Biol. N.Y.*, 1944, **56**, 178.

[32] C. P. Miller and A. Z. Foster, *ibid.*, p. 205.

[33] G. L. Hobby and M. H. Dawson, *ibid.*, p. 181.

[34] C.-E. A. Winslow and H. H. Walker, *Bact. Rev.*, 1938, **3**, 147.

[35] W. W. Smith. *Proc. Soc. Exp. Biol. N.Y.*, 1944, **56**, 238.

[36] D. P. Evans and A. G. Fishburn, *Quart. J. Pharm.*, 1943, **16**, 201.

[37] R. C. Jordan and S. E. Jacobs, *J. Hygiene*, 1944, **43**, 275.

[38] O. Rahn, *Biodynamica*, 1943, **4**, 81.

[39] E. I. Valko and A. S. DuBois, *J. Bact.*, 1944, **47**, 15.

liminary reversible one and later irreversible changes associated with death. The reversible one is considered to be the attachment of the agent to the cell and was shown to have some of the characters of ionic exchange; the action was reduced by the additional presence of less toxic cations. Such antagonism was effective against only limited concentrations of toxic cation. Similar phases in the action of other bactericides have been proposed;[36] here also the second phase was considered to be fundamentally different and to consist in denaturation or precipitation of the bacterial protein. The activities of antiseptics at different pH have been related to the concentrations of ionised and undissociated molecules; undissociated and not ionised benzoic, salicylic, and sulphurous acids were found antiseptic.[40] Œstrogens and related compounds are bactericidal,[41 42] but optimal antibacterial activity is not shown by members of greatest œstrogenic activity.[43] Propamidine is bactericidal as well as bacteriostatic to staphylococci [44] and to *Escherichia coli* [45] and both effects are antagonised by lecithin.[45]

II. *Biochemical Effects.*

(a) *Upon Energy-yielding Processes.*—Evidence has been collected [46] suggesting a correlation of the inhibitions of bacterial respiration or anaerobic carbon dioxide production, with inhibition of growth, by sulphonamides. The respiratory inhibition is only partial (and by some investigators has been reported absent) at concentrations of sulphonamides which are completely bacteriostatic. To affect glycolysis or respiration of streptococci in the presence of glucose and a few other substrates, pantoyltaurine is required in much greater preponderance over pantothenate than is required for it to inhibit growth;[8] these metabolic inhibitions also are relatively small or may be absent. Oxidation of amino-acids by *Escherichia coli* is inhibited by low concentrations of propamidine and is more sensitive to the compound than is oxidation of glucose.[47] The inhibitions are markedly increased by adding the inhibitor before the substrate, and by increase in pH.[48] The activity of antimalarials in inhibiting oxygen uptake of malarial parasites is correlated with their therapeutic efficacy.[49]

(b) *Upon Metabolism of Vitamin-like Compounds.*—The system at which sulphonamides and *p*-aminobenzoate are believed to interact has not yet been specified biochemically, but further interpretations of actions of sulphonamides in terms of their competing with *p*-aminobenzoate for enzymes

[40] O. Rahn and J. E. Conn, *Ind. Eng. Chem.*, 1944, **36**, 185.
[41] G. H. Faulkner, *Lancet*, 1943, **245**, 38.
[42] B. Heinemann, *J. Lab. Clin. Med.*, 1944, **29**, 254.
[43] G. Brownlee, F. C. Copp, W. M. Duffin, and I. M. Tonkin, *Biochem. J.*, 1943, **37**, 572.
[44] W. R. Thrower and F. C. O. Valentine, *Lancet*, 1943, **244**, 133.
[45] W. O. Elson, *J. Biol. Chem.*, 1944, **154**, 717.
[46] R. J. Henry, *Bact. Rev.*, 1943, **7**, 175.
[47] F. Bernheim, *Science*, 1943, **98**, 223.
[48] F. Bernheim, *J. Pharm. Exp. Ther.*, 1944, **80**, 199.
[49] S. R. Christophers, *Trans. Faraday Soc.*, 1943, **39**, 333.

have been given.[50, 51] Increased synthesis of *p*-aminobenzoate has been found to be associated with development of sulphonamide-resistance in staphylococci.[52] By training certain strains of *Corynebacterium diphtheriæ* to synthesise pantothenate, strains resistant to pantoyltaurine were produced in the absence of that compound and of any other inhibitor;[53] but not all drug resistance is by synthesis of specific antagonists.[19] The system through which pantoyltaurine inhibits streptococcal growth has to some extent been characterised [54] and its functioning is associated with pantothenate-inactivation. In the preparations studied, the pantothenate metabolism required a concurrent energy-yielding process such as glycolysis. The pantothenate metabolism, but not glycolysis, was inhibited by concentrations of pantoyltaurine even lower than those affecting growth and the activities of a series of pantothenate analogues in inhibiting growth were correlated with their activities in inhibiting pantothenate-inactivation. A bacterial degradation of riboflavine is inhibited by structurally related compounds but occurs independently of a process such as glycolysis and its inhibition does not affect growth.[55]

III. *Chemical or Physical Effects.*

Analyses of sulphonamide action, the effect of pH upon it, and its antagonism, base these processes upon reversible combination of the drug, antagonist, or their ions with enzymes in accordance with the law of mass action.[50, 51, 56] The bulk of the *p*-aminobenzoate of preformed organisms is not, however, displaced by bacteriostatic concentrations of sulphanilamide;[57] the equilibria may obtain during or before *p*-aminobenzoate assimilation. Similar lack of displacement of pantothenate by pantoyltaurine has been observed.[57] Correlation of the action of drugs with properties which they exhibit apart from biological systems has been reported [36, 50, 58] and reviewed.[59, 60]

IV. *Chemotherapeutic Mechanisms.*

The year's findings have shown the multiplicity of types of antibacterial action exhibited by chemotherapeuticals. The connection of these with chemotherapeutic activity *in vivo* is established only in certain cases and in others would not be expected to be very close. Discussion of such connections (chemotherapeutic "mechanisms") is beyond the scope of the present account, but it will be seen that evidence for such connections is

[50] W. D. Kumler and T. C. Daniells, *J. Amer. Chem. Soc.*, 1943, **65**, 2190.
[51] I. M. Klotz, *ibid.*, 1944, **66**, 459.
[52] M. Landy, N. W. Larkum, E. J. Oswald, and F. Streightoff, *Science*, 1943, **97**, 295.
[53] H. McIlwain, *Brit. J. Exp. Path.*, 1943, **24**, 212.
[54] H. McIlwain and D. E. Hughes, *Biochem. J.*, 1944, **38**, 187.
[55] J. W. Foster, *J. Bact.*, 1944, **48**, 97.
[56] F. H. Johnson, H. Eyring, and W. Kearns, *Arch. Biochem.*, 1943, **3**, 1.
[57] H. McIlwain, *Proc. Biochem. Soc.*, 1944, **38**, viii.
[58] A. Albert and R. Goldacre, *J.*, 1943, 454.
[59] A. Albert, *Australian J. Sci.*, 1944, **6**, 137.
[60] W. S. Gledhill, *ibid.*, p. 170.

most fully provided in the cases of pantoyltaurine and the sulphonamides, by observations *in vivo* and of categories I (*b*), II (*a*), II (*b*), and III. Other compounds are of radically different mode of action and one compound may act in more than one way.[61] As the normal life of organisms involves a working together of processes which include all the above categories, many other means can be envisaged for their disturbance. H. McI.

F. Dickens.
H. McIlwain.
A. Neuberger.
F. W. Norris.
J. R. P. O'Brien.
F. G. Young.

[61] C. E. Hoffmann and O. Rahn, *J. Bact.*, 1944, **47**, 177.

ALTHOUGH this Report deals with only four topics, the first three sections illustrate a large variety of current analytical trends.

I. *Zinc.*

No spectacular advances in the analytical chemistry of zinc have been recorded, but there has been fairly steady development, stimulated to some extent by recognition of the biological importance of the element.

(1) *Separations and Qualitative Tests.*—A procedure is given [1] for determination of zinc and other metals in foodstuffs, by precipitation with hydrogen sulphide at pH 8 after a wet ashing process. The precipitate is dissolved, and iron, etc., precipitated with ammonia. After acidification, copper is separated with hydrogen sulphide and zinc determined with 8-hydroxyquinoline.

Other authors [2, 3] write on the use of hydrogen sulphide. McLellan points out that in 1 : 19 hydrochloric acid there is appreciable coprecipitation of zinc with Group 2 metals. Laws uses a formic acid buffer for separation of zinc from many other metals in light and in nickel alloys, but S. A. Coleman and G. B. L. Smith [4] prefer to use citric acid as a buffer. E. A. Ostroumov [5] says that post-precipitation of cobalt on zinc sulphide can be prevented by the use of acraldehyde in the solution and that filter-paper pulp, unless previously treated with buffer solution, materially increases the contamination. C. Zöllner [6] stated that in absence of hydrochloric acid, cadmium is quantitatively precipitated by hydrogen sulphide from a solution containing 15 ml. of concentrated sulphuric acid per 100 ml.; the solution is boiled, and allowed to cool while the gas is passing. There are also several references [7, 8, 9] to separation of cadmium from zinc by means of aluminium foil or powder, which precipitates cadmium from a somewhat acid hydrochloric acid solution. It would appear best to separate most of the cadmium in this way, and recover the remainder from the filtrate by means of hydrogen sulphide.

A novel method is put forward by C. Mahr and H. Ohle.[10] If to a solution containing cadmium and zinc, thiourea and Reinecke's salt are added in the cold, cadmium is quantitatively precipitated as

$$Cd(CS{\cdot}N_2H_4)_2,[Cr(CNS)_4(NH_3)_2]_2,$$

leaving all the zinc in the filtrate. Complex thiocyanates have been studied; *e.g.*, W. C. Vosburgh, G. Cooper, W. J. Clayton, and H. P. Pfann [11] state that

[1] J. H. Hamence, *Analyst*, 1937, **62**, 18.
[2] G. McLellan, *J. Assoc. Off. Agric. Chem.*, 1941, **24**, 728.
[3] E. Q. Laws, *Analyst*, 1941, **66**, 54.
[4] *Ind. Eng. Chem. Anal.*, 1941, **13**, 377.
[5] *Ann. Chim. anal.*, 1937, **19**, 145.
[6] *Z. anal. Chem.*, 1938, **114**, 8.
[7] J. J. Lurie and V. F. Neklyntina, *Zavod. Lab.*, 1936, **5**, 87.
[8] E. I. Nikitina, *ibid.*, 1939, **8**, 1172.
[9] F. E. Townsend and G. N. Cade, *Ind. Eng. Chem. Anal.*, 1940, **12**, 163.
[10] *Z. anal. Chem.*, 1937, **109**, 1.
[11] *Ind. Eng. Chem. Anal.*, 1938, **10**, 393.

for precipitation of $ZnHg(CNS)_4$, 100 ml. of solution may contain not more than 2·5 g. of nitric or 5 g. of sulphuric acid. The precipitate can be weighed after drying at 105°, but W. Hoffman and G. B. Thackray [12] prefer to titrate it by R. Lang's procedure,[13] or to use potassium iodate. In analysis of brass or bronze they precipitate copper as $Cu_2(CNS)_2$ as usual, and to the filtrate add $(NH_4)_2Hg(CNS)_4$ to precipitate the zinc salt. Similarly, a micro-method for zinc in ores [14] precipitates $ZnHg(CNS)_4$ and titrates it with potassium iodate. Iron may be masked with potassium fluoride, citric acid, or (best) phosphoric acid, but large amounts of iron or aluminium prevent the precipitation.

It is stated [15] that β-naphthaquinoline in 0·1N-sulphuric acid and potassium thiocyanate give a very sensitive qualitative reaction for zinc. Characteristic crystals appear from a 2N-acid solution containing more than 1 part of zinc per 200,000 parts. Less than 0·5% of cadmium does not interfere, but numerous other metals do.

Forty-five proposed qualitative reagents are tabulated by P. Wenger and R. Duckert.[16] They recommend pyridine and potassium bromide in neutral solution (nickel also reacts), potassium ferrocyanide in acid solution (cadmium also reacts), $K_2Hg(CNS)_4$ and cobalt chloride (iron and manganese interfere). As " spot " tests they recommend metanil-yellow with potassium ferricyanide, and also dithizone. Benzoin in presence of alkali and magnesium ions is a highly specific reagent for zinc,[17] giving a compound with a vivid green fluorescence in U.V. light. Only bismuth, boron, and antimony interfere and the test will detect 1 μg. of zinc in 1 ml. of solution.

(2) *Use of Organic Reagents.*—Anthranilic acid, first proposed by H. Funk and M. Ditt,[18] was thoroughly investigated by R. J. Sherman, J. H. F. Smith, and A. M. Ward,[19] who dealt with the solubility of the Zn, Cd, Cr, Ni, and Cu salts : the solubility of the zinc salt is increased by sodium acetate, but even in absence of salts, results are somewhat low. Bromination of the precipitate with standard bromide–bromate solution to the tribromo-compound is preferred, but C. W. Anderson [20] weighs the precipitate, which is formed in acetic acid solution, salts being absent. Preliminary separation of the zinc is essential, and P. Wenger [21] points out that the temperature of precipitation is unimportant.

Quinaldinic acid has also received attention. R. R. Ray and M. K. Bose [22] claim very good results by a micro-precipitation with sodium quinaldinate from a hot solution faintly acid with acetic acid. The precipitate is dried at 125°. A thorough investigation was made by R. J. Shennan,[23] who showed that the reagent completely precipitates copper between pH

[12] *Analyst*, 1941, **66**, 321.
[13] *Z. anal. Chem.*, 1929, **79**, 161.
[14] J. J. Lurie and L. A. Philippova, *Zavod. Lab.*, 1939, **8**, 1047.
[15] E. B. Sandell, D. B. Wishnick, and E. L. Wishnick, *Microchim. Acta*, 1938, **3**, 204.
[16] *Helv. Chim. Acta*, 1942, **25**, 400.
[17] C. E. White and M. H. Neustadt, *Ind. Eng. Chem. Anal.*, 1943, **15**, 599.
[18] *Z. anal. Chem.*, 1933, **91**, 332.
[19] *Analyst*, 1936, **61**, 395.
[20] *Ind. Eng. Chem. Anal.*, 1943, **15**, 367.
[21] *Helv. Chim. Acta*, 1942, **25**, 1499.
[22] *Mikrochem.*, 1935, **17**, 11.
[23] *Analyst*, 1939, **64**, 14.

2·5 and 6·9, cadmium from pH 3·9 to 7·2, and zinc at pH 2·3—6·5. No separations are possible, and acetates have a marked effect on the solubility, but P. R. Rây and T. C. Sarkar [24] showed that if the copper is reduced to cuprous in the presence of thiourea, it is masked and does not interfere. A similar process can be used in presence of mercury.

5-Nitroquinaldinic acid precipitates zinc from feebly acid solutions, and has been made the basis of a colorimetric method.[25] Most other metals interfere. Another reagent with which most common anions and cations interfere, and which would therefore appear to be of very limited use, is tetraphenylarsonium chloride.[26] Salicylaldoxime also precipitates zinc. L. P. Biefield and W. B. Ligett [27] give the pH at which it precipitates zinc, copper, and lead. T. G. Pearson [28] says it is not a suitable reagent, and J. H. Flagg and N. H. Furman [29] describe conditions under which it could be used. A really useful (but not selective) reagent, however, is 8-hydroxyquinoline, probably the only one of these reagents which is often used. In a most important paper,[30] the authors examined a number of " oxinates " by *X*-ray diffraction, and showed that the dihydrated oxinates of zinc and magnesium (as of copper and iron) are amorphous, a fact of very great importance in explaining the difficulty of many separations which would appear to be possible with this reagent. They also showed that from solutions containing both ions, the precipitates are solid solutions. This did not confirm other work [31] which explained the same phenomenon by adsorption. An earlier paper [32] on separations states that zinc can be separated from manganese in two precipitations at pH 5—6, from iron and bismuth in presence of tartrates at pH 8, from arsenic and antimony, but not from cobalt or nickel, by this reagent.

(3) *Colorimetric and Micro-procedures.*—Although diphenylthiocarbazone (" dithizone ") is one of the least specific of reagents, it has been useful in the determination of really small amounts of zinc. It was at first applied rather tentatively, but the investigations of many recent authors, in particular the members of the American " Association of Official Agricultural Chemists," have rendered it most serviceable in an enormous number of ways. The first qualitative use of the reagent appears to have been by H. Fischer,[33] and later with G. Leopoldi,[34] he showed how, in a barely acid solution, most metals could be masked with thiosulphate, rendering the reagent practically specific for zinc, potassium cyanide being added if cobalt or palladium was present. In the first application of the reagent to foods,[35] a chloroform

[24] *Mikrochem.*, 1939, **27**, 64.
[25] W. L. Lott, *Ind. Eng. Chem. Anal.*, 1938, **10**, 331.
[26] H. H. Willard and G. M. Smith, *ibid.*, 1939, **11**, 269.
[27] *Ibid.*, 1942, **14**, 359.
[28] *Z. anal. Chem.*, 1938, **112**, 179.
[29] *Ind. Eng. Chem. Anal.*, 1940, **12**, 663.
[30] R. C. Chirnside, C. F. Pritchard, and M. P. Rooksby, *Analyst*, 1941, **66**, 339.
[31] H. V. Mayer and W. J. Remington, *Ind. Eng. Chem. Anal.*, 1938, **10**, 212.
[32] C. Cimerman and P. Wenger, *Mikrochem.*, 1939, **27**, 76.
[33] *Ibid.*, 1930, **8**, 319.
[34] *Z. anal. Chem.*, 1936, **107**, 241.
[35] N. D. Sylvester and E. B. Hughes, *Analyst*, 1936, **61**, 734.

solution was used to extract a solution of the ash buffered at pH 4·5 with ammonium acetate. Zinc (with bismuth and cadmium if present) was extracted from the chloroform solution with diluted hydrochloric acid and finally titrated either with potassium ferrocyanide or, after addition of potassium ferricyanide and iodide, with N/500-thiosulphate solution.

E. A. Coakhill [36] improved upon P. L. Hibbard's colorimetric process.[37] In the analysis of commercial lead, the bulk of the lead (from a 2—5 g. sample) is removed as sulphate, and most of what remains by hydrogen sulphide in a solution acid enough to prevent precipitation of zinc sulphide. Thioglycollic acid prevents reaction of lead with dithizone, so a just ammoniacal solution is extracted with a chloroform solution of both reagents until no further change of colour takes place. A " blank " solution is now similarly treated, and standard zinc solution run in until the colours match. A very important colorimetric application is described by F. H. Vogelenzang [38] in the determination of zinc in blood, etc. All reagents contain zinc and should be extracted by the dithizone reagent before use; glass often contains zinc and such glass must not be used. Zinc and copper are extracted together from a weakly ammoniacal solution, zinc stripped out with 2N-hydrochloric acid, and finally collected in a carbon tetrachloride solution of the reagent; after filtration, the solution is diluted to known volume, and the red colour measured in a Pulfrich photometer. It has a maximum extinction at 5250 A., and obeys Beer's law.

Several investigators used dithizone to isolate the zinc, and then determined it in some other way,[39] but generally, progress has been in pure colorimetry, although E. B. Sandell [40] proceeds by " extraction titration " at pH 4·1, sodium thiosulphate masking other elements in soils, and P. L. Hibbard,[41] having isolated the zinc compound, titrates it with bromine.

H. J. Wichman [42] points out that sodium diethyldithiocarbamate masks almost all elements except zinc, and that photometry at the proper wavelength enables zinc to be determined without removing excess of reagents. This important modification is being extended by R. A. Caughley, G. B. Holland, and W. S. Ritchie,[43] and the last two authors [44] emphasise the presence of zinc in most reagents and in glass, and include a valuable table giving the reaction of many metals in 0·02N-ammonia or 0·02N-hydrochloric acid to dithizone, " carbamate," and both together; in 0·02N-ammonia, this combination is specific. The analytical procedure is typical of this kind of technique. The extract of the plant ash or the like, containing ammonia and ammonium citrate, is treated with a carbon tetrachloride solution of dithizone, until all reacting metals are extracted. The tetrachloride layer is then shaken with 0·02N-hydrochloric acid, which removes

[36] *Analyst*, 1938, **63**, 800.
[37] *Ind. Eng. Chem. Anal.*, 1937, **9**, 127.
[38] *Pharm. Weekblad*, 1939, **76**, 89.
[39] *E.g.*, J. H. Peckman and J. E. Menshing, *J. Assoc. Off. Agric. Chem.*, 1937, **20**, 627.
[40] *Ind. Eng. Chem. Anal.*, 1937, **9**, 464.
[41] *Ibid.*, 1938, **10**, 615.
[42] *J. Assoc. Off. Agric. Chem.*, 1938, **21**, 197.
[43] *Ibid.*, p. 204.
[44] *Ibid.*, 1939, **22**, 333.

lead, zinc, cobalt, silver, and cadmium, leaving copper and mercury in the solvent layer. The aqueous layer is made 0·02N with ammonia, buffered with ammonia citrate, and extracted with carbon tetrachloride–dithizone after addition of "carbamate," which brings zinc into the solvent layer. Unfortunately, in presence of "carbamate" extraction of zinc is not quite complete,[45] and the partition ratio between the tetrachloride dithizone phase and the aqueous phase is influenced by pH and concentration of the reagents in the two phases. By keeping these factors constant, accurate results can be obtained, preferably by finally measuring extinction at 5400 A., a more convenient process than extracting excess dithizone with 0·01N-ammonia, leaving zinc dithizone in the carbon tetrachloride phase. For zinc in soils (and also copper) G. D. Sherman and J. S. McHargue[46] proceed somewhat similarly to Holland and Ritchie, but before the final extraction of zinc, they mask other metals (*e.g.*, lead) with sodium thiosulphate.

Finally, di-β-naphthylthiocarbazone is proposed as a superior reagent with many features similar to dithizone.[47] Transmission diagrams are given, and the reagent quantitatively extracts zinc from solutions of pH $> 7{\cdot}5$, even when "carbamate" is present. Full instructions are given for the elimination of interferences. If less than 0·05 mg. is present, and no cadmium, photometry of the carbon tetrachloride solution is recommended, but if more zinc or zinc and cadmium are present, the tetrachloride solution is "stripped" with 0·2N-hydrochloric acid and polarographed from $-0{\cdot}3$ to $1{\cdot}3$ v., the zinc step taking place at $-1{\cdot}1$ v. The addition of cadmium (0·6 mg.) as an "internal standard" is a useful device, or after polarography, a known amount of zinc is added, and from the heights of the zinc steps in the two polarograms, the zinc content of the sample is calculated.

(4) *Miscellaneous Developments.*—Polarography as a micro-method may be far simpler than colorimetric methods. J. F. Reed and R. W. Cummings[48] describe the polarographic determination in soils and plant ash. None of the constituents of plant ash interferes when the pH of the solution is 4·6, and the solution is 0·025N with respect to thiocyanate ions. On a 1-g. sample, zinc may be determined with an error of $\pm 5\%$ when present between 0·5% and 5 parts per million. The determination of zinc polarographically in paints[49] and in brass plate[50] is also described, the latter determination being made in 20 minutes. A publication by the British Aluminium Company, Ltd., discusses analysis of aluminium (and magnesium) alloys by means of the polarograph and the spectrograph.[51]

R. T. O'Connor[52] describes a spectrographic method for traces of zinc in fertilisers, beryllium being added to act as an internal standard, and the

[45] H. Cowling and E. J. Miller, *Ind. Eng. Chem. Anal.*, 1941, **13**, 145.
[46] *J. Assoc. Off. Agric. Chem.*, 1942, **25**, 510.
[47] J. Cholak, D. M. Hubbard, and R. E. Burkey, *Ind. Eng. Chem. Anal.*, 1943, **15**, 754.
[48] *Ibid.*, 1940, **12**, 489. [49] B. M. Abraham and R. S. Huffman, *ibid.*, p. 656.
[50] W. P. Tyler and W. B. Brown, *ibid.*, 1943, **15**, 520.
[51] Publication 401, 1943. [52] *Ind. Eng. Chem. Anal.*, 1941, **13**, 597.

Be line 2348 A. being used. Zinc can be determined between 0·0008% and about 1%. E. J. Magaziner and N. A. Zventitzki [53] suggest striking the arc between a copper electrode and the sample, using for comparison the lines Cu 2824 and Zn 2756, but if lead is also present Cu 2883 is used.

There are a few references to electrodeposition. In deposition from neutral citrate solution, very many metals interfere,[54] but excellent results are obtained by electrolysis at 2 amps. from a slightly alkaline solution on to a copper-plated platinum electrode, after a preliminary separation; [55] the method, for alloys, is very rapid. A. Cohen [56] recommends electrolysis from alkaline tartrate solution in analysis of aluminium alloys, and also discusses the mercury thiocyanate precipitation. He emphasises that by the usual procedure of dissolving the alloy in sodium hydroxide, some zinc always remains insoluble. One or two papers discuss the use of complex cyanides in alloy analysis. C. C. Casto and A. J. Boyle,[57] in analysis of magnesium alloys, remove manganese and copper, add citrate and dilute sulphuric acid, and proceed by Lang's method.[13] If less than 0·5% of zinc is present, a larger sample must be used. In the ferricyanide titration, *p*-ethoxychrysoidine [58] and *o*-anisidine [59] are recommended as indicators. In brass analysis, by heating to 800—850° in a vacuum (10^{-4} mm.), zinc (and also lead) are distilled off, and the efficiency is greater the higher the proportion of zinc.[60] Finally, three papers on commercial analysis for zinc are noteworthy. Zinc oxalate is found to be insoluble in 70% acetic acid if ammonium chloride is absent; [61] the precipitate is washed twice in a centrifuge tube with 10% acetic acid, and the oxalate finally titrated with permanganate (nickel, lead, and copper interfere). L. G. Miller, A. W. Boyle, and R. B. Neill [62] dissolve magnesium alloys in dilute hydrochloric acid, adding lead if necessary to prevent solution of copper, then add a little ferricyanide to mask any iron usually present, make the solution N/1 in respect to hydrochloric acid, add excess of ferrocyanide solution, and after filtration, back-titrate excess with ceric sulphate. The method is very rapid and accurate to 1—6%. Zinc in magnesium alloys is also dealt with by S. Weinberg and T. F. Boyd.[63] The sample is dissolved in diluted sulphuric acid, a large excess of ammonium chloride and tartaric acid added, then excess of ammonia, and the solution electrolysed for 20 minutes at 2 amps. If significant quantities of Group 2 metals are present, they must be removed by means of hydrogen sulphide. Apart from this case, the determination is complete in 25 minutes. H. N. W.

[53] *Zavod. Lab.*, 1940, **9**, 992.
[54] R. Winchester and L. F. Yntema, *Ind. Eng. Chem. Anal.*, 1937, **9**, 254.
[55] G. H. Osbourne, *Analyst*, 1941, **66**, 412.
[56] *Helv. Chim. Acta*, 1943, **26**, 75.
[57] *Ind. Eng. Chem. Anal.*, 1943, **15**, 624.
[58] W. P. Tyler, *ibid.*, 1942, **14**, 114.
[59] H. F. Frost, *Analyst*, 1943, **68**, 51.
[60] W. P. Treadwell and G. Frey, *Helv. Chim. Acta*, 1944, **27**, 42.
[61] P. J. Ewing and J. C. Lamkin, *Ind. Eng. Chem. Anal.*, 1944, **16**, 194.
[62] *Ibid.*, p. 256. [63] *Ibid.*, p. 460.

II. *Arsenic.*

The following report is divided into sections, each dealing with the application of a particular reaction or technique.

The Gutzeit Method.—A thorough investigation [1] discusses various errors to which the process is subject. Since zinc varies in activity, if dense massive zinc be used, the temperature of evolution of arsine should be higher (40—60°); even in the presence of stannous chloride, all the arsine is not evolved from quinquevalent compounds, and reduction by sulphurous acid on a water-bath is recommended, followed by brief boiling, before evolution of arsine, but addition of a little potassium iodide (as a catalyst) and then stannous chloride, following the A.O.A.C. method, is equally effective.

A very important paper [2] describes the determination of minute amounts, as little as 0·1 μg., with a probable error of less than 5% and sensitive to 0·01 μg. The author uses thin cotton threads impregnated with mercuric chloride as absorbents; they are enclosed in capillary tubes, into which they just fit. All conditions must be rigidly standardised, including temperature of evolution and of the absorption tubes. Quinquevalent arsenic is reduced by potassium bisulphite, stated to be preferable to the iodide, and a little ferrous salt as catalyst. The stains are developed by ammoniacal silver nitrate solution and measured with a Vernier caliper. This technique has been further studied.[3]

L. Truffert [4] returns to the reaction of arsine on a silver salt in determining arsenic in wines. Platinised zinc and sulphuric acid are used to generate hydrogen, and the gases are passed first through potassium hydroxide solution. The paper, used in strips, is coated with silver citrate, and is stable in the dark; 1—30 μg. can be estimated.

The Sub-committee of the Institute of Brewing [5] considers two methods of estimating the evolved arsine—those of Marsh and Gutzeit. In the latter, discs are preferred to strips, and mercuric bromide is about twice as sensitive as the chloride, but preparation of the paper, which will not keep, needs greater care. The lowest limits are stated as 0·001 mg. for the Marsh–Berzelius method, and 0·0004 mg. for the Gutzeit process. Despite the impermanent nature of the stains, the latter is preferred. For hops, malt, beer, sugar, and finings, methods of preparing the solutions which do not require destruction of organic matter are described. Coal is ashed with equal weights of magnesia and potassium permanganate, and in all cases potassium iodide and sodium sulphite are used to reduce As^V. Alternatively,[6] coal or coke is ashed with a mixture of magnesia, sodium carbonate, and potassium nitrate; after solution of the residue As^V is reduced by sulphite and determined by the mercuric bromide modification of Gutzeit's method. A con-

[1] W. A. Davis and J. G. Maltby, *Analyst*, 1936, **61**, 96.
[2] A. E. How, *Ind. Eng. Chem. Anal.*, 1938, **10**, 226.
[3] E. Cahill and L. Walters, *ibid.*, 1942, **14**, 90.
[4] *Ann. Falsif.*, 1938, **31**, 73. [5] *J. Inst. Brewing*, 1938, **44**, 359.
[6] D.S.I.R. Fuel Research Paper No. 44, Oct. 28th, 1940.

tinuous apparatus is described [7] in which hydrogen is evolved from a long cadmium cathode, from an electrolyte containing sulphuric acid and hydroxylamine; from time to time a sample is introduced below the cathode, flows up past it to the platinum anode, and so to waste. The evolved hydrogen is passed through a heated glass tube. As most samples examined are practically free from arsenic, they can very rapidly be dealt with in this apparatus: a contaminated sample is immediately recognised and the mirror can be compared with standards.

Less than 0·1 mg. of selenium is stated to have no effect on arsine methods.[8]

The estimation of arsenic in foodstuffs, etc., contaminated with war gases [9, 10] cannot be suitably summarised. G. Taylor and J. H. Hamence [11] state that if zinc alloyed with 0·3% of copper is used, the whole of the arsenic is liberated without the use of sulphites in the presence of "stannated" hydrochloric acid in the Gutzeit method.

Reduction by Means of Hypophosphite.—This method continues to receive attention. White metals are dissolved in hydrochloric acid in presence of bromine, arsenic precipitated with hypophosphite, redissolved, reduced with sodium sulphite after addition of sulphuric acid, and finally titrated with bromate solution. Antimony does not interfere.[12] As internal indicators for the bromate titration, Bordeaux, naphthol blue-black, and brilliant Ponceau-SR are recommended.[13] If reduction is carried out in the solution at 90°, it is so rapid that there is no loss of arsenic.[14] The precipitated metal is dissolved in N/50-ceric sulphate solution, excess being titrated with N/200-arsenious oxide solution, with osmic acid as catalyst. Antimony and tin do not interfere, and the method is valid for 0·1—2 mg. of arsenic. W. J. Agnew [15] proceeds similarly, but uses N/100-potassium dichromate to dissolve the metal, and back-titrates excess with N/100-ferrous sulphate. The effect of selenium and tellurium is discussed; H. J. G. Challis [16] points out that these elements are also precipitated by hypophosphite but below 50° are precipitated free from arsenic. After filtration, arsenic can be precipitated by more hypophosphite on boiling, but B. S. Evans [17] says that this is only true of "traces" of arsenic. He precipitates the three elements (say, in analysis of copper) together by hypophosphite, redissolves them, and precipitates selenium with potassium iodide and tellurium with sulphur dioxide, leaving arsenic in solution to be precipitated later with hypophosphite as usual. The process is applied [18] to organic arsenicals, after wet oxidation

[7] H. C. Lockwood, *Analyst*, 1939, **64**, 657.
[8] Fuel Research Board: Report for year ending March 31st, 1939.
[9] H. A. Williams, *Analyst*, 1941, **66**, 228.
[10] A. McM. Taylor and W. J. Stainsby, *ibid.*, p. 233. [11] *Ibid.*, 1942, **67**, 12.
[12] C. W. Anderson, *Ind. Eng. Chem. Anal.*, 1937, **9**, 569.
[13] G. F. Smith and R. L. May, *ibid.*, 1941, **13**, 460.
[14] J. M. Kolthoff and E. Andrew, *ibid.*, 1940, **12**, 177.
[15] *Analyst*, 1943, **68**, 111. [16] *Ibid.*, 1941, **66**, 58. [17] *Ibid.*, 1942, **67**, 346.
[18] H. A. Sloviter, W. M. McNabb, and E. C. Wagner, *Ind. Eng. Chem. Anal.*, 1942, **14**, 516.

with sulphuric and nitric acids, but is not applicable [19] to cacodyl derivatives, which should be decomposed with potassium bisulphate and sulphuric acid; after solution is complete a version of the hypophosphite process [20] is used.

There are two mentions of a curious process in which the reduced metal is kept in a colloidal suspension. J. V. Harispe [21] applies this to the semi-micro-analysis of urine: after destruction of organic matter, hydrochloric acid, hypophosphite, and a dilute solution of potassium silicate (protective colloid) are added, and after the mixture has been heated on a water-bath, the colour is compared with standards. J. Thuret [22] says that the arsenic slowly flocculates even in presence of stabilisers, and recommends a standard solution containing borax and colophony, which has the same appearance and remains stable for one month.

Distillation Methods.—For other than traces, distillation of arsenic trichloride and titration of the distillate continues to be largely used. The determination in wood of arsenic added as preservative is described: [23] after wet oxidation with sulphuric and nitric acids, the latter acid is removed by repeated evaporation, and the trichloride distilled as usual. The distillate is oxidised with nitric acid, excess of this removed, As^V reduced in acid solution with potassium iodide, and finally titrated with iodine in presence of excess of sodium bicarbonate. For smaller amounts, a modification of Gutzeit's process is used on aliquots of the distillate. An important paper [24] describes the quantitative separation of arsenic, antimony, and tin by fractional distillation of the aqueous solution of the chlorides. For arsenic in pyrites, [25] the mineral is fused with sodium carbonate and peroxide, the mass acidified with hydrochloric acid, and the trichloride distilled, hydrazine and potassium bromide being used as reducing agents; the distillate is titrated with bromate. In the analysis of soils which have been treated with lead arsenate, L. Koblitsky [26] oxidises interfering organic matter if necessary with 30% hydrogen peroxide, and distils after reduction with hydrazine and potassium bromide. H. N. Wilson [27] determines total arsenic in glass by fusion with sodium hydroxide, acidification of the melt with hydrochloric acid, in such a way that silica is not precipitated, distillation with this acid, hydrazine and bromide being the reducing agents, and titration of the arsenious chloride in the distillate (which is of the correct acidity) by N/200-potassium iodate. The method, applicable to 0·02% arsenious oxide and upwards, is rapid and accurate. V. Dimbleby [28] discusses the same process.

Colorimetric Processes.—The most interesting development in arsenic determinations is the growth in popularity of these processes, in all of which

[19] V. Levine and W. M. McNabb, *ibid.*, 1943, **15**, 76.
[20] Ref. 18.
[21] *J. Pharm. Chim.*, 1939, **30**, 58.
[22] *Ann. Falsif.*, 1939, **32**, 328.
[23] Commonwealth of Australia. Division of Forest Products, Reprint No. 29, 1936.
[24] J. A. Scherrer, *Bur. Stand. J. Res.*, 1938, **21**, 95.
[25] T. A. Fedorkin, *Zavod. Lab.*, 1940, **9**, 1324.
[26] *J. Assoc. Off. Agric. Chem.*, 1939, **22**, 680.
[27] *Analyst*, 1943, **68**, 361. [28] *Glass Review*, 1943, **19**, 120.

arsenomolybdate is formed and reduced, the blue colour being a function of the arsenic concentration. The arsenic is first isolated from interfering substances by distillation as chloride or hydride. Organic matter in must or wine [29] is destroyed with nitric and sulphuric acids and hydrogen peroxide, arsenic distilled as chloride, and the distillate evaporated to dryness with nitric acid. The arsenic, now quinquevalent, is colorimetrically determined by O. Zinzadze's reagent,[30] preferably with a Zeiss photometer. The extinction is proportional to the arsine content from 0·01 to 0·8 mg. J. A. Scherrer [31] describes a superior form of Zinzadze's reagent. D. M. Hubbard [32] distils the chloride in a current of carbon dioxide, oxidises the distillate as above, and obtains molybdenum-blue by a molybdate–hydrazine sulphate reagent, reacting at 70—75° for 30 minutes. The colour is stable for 24 hours. The maximum absorption is in the near infra-red at 8400 A., which gives twice the optical density of that at 7400 A.[33] An elegant oxidation of the chloride is effected [34] by distilling it in a special apparatus, so that the vapours pass through a few ml. of potassium iodate solution, whereby it is oxidised, steam and hydrogen chloride passing on to be condensed and returned to the flask. The process is very rapid.

Two methods avoid distillation by extracting the acid arsenious solution with a carbon tetrachloride solution of sodium ethylxanthate. A. Klein and F. A. Vorhes [35] evaporate the extract to dryness and oxidise the residue with bromine water, then applying Zinzadze's reagent. T. B. B. Crawford and I. D. E. Storey [36] extract inorganic arsenite from blood, urine, etc., by means of the xanthate (three extractions) and then proceed as in the foregoing or by means of G. A. Levvy's method.[37]

Several papers deal with the evolution, oxidation, and colorimetric determination of arsine, the arsenomolybdate being used. E. B. Sandell [38] passes the arsine and hydrogen (from 20—30 mesh zinc) through mercuric chloride and potassium permanganate, evolution of arsine being complete in 30 minutes; the solution is filtered and treated by Hubbard's method.[32] The procedure is suitable for 1—10 μg. of arsenic. M. B. Jacobs and J. Nagler [39] oxidise the arsine by hypobromite, and R. Milton and W. B. Driffield [40] by iodine and sodium hydrogen carbonate solution. In all cases careful adherence to standard quantities of reagents is necessary, and also to exact control of acidity, as is always the case for quantitative formation and reduction of molybdenum complex ions. In controlled conditions, the blue colour obeys Beer's law from 0·001 to 0·1 mg. of arsenious oxide per 10 ml.

Arsenic can be colorimetrically determined in lead without distillation

[29] J. Burkard and B. Wallhorst, *Z. Unters. Lebensm.*, 1935, **70**, 308.
[30] *Ind. Eng. Chem. Anal.*, 1935, **7**, 227. [31] *Bur. Stand. J. Res.*, 1938, **21**, 95.
[32] *Ind. Eng. Chem. Anal.*, 1941, **13**, 915.
[33] J. A. Stultzaberger, *ibid.*, 1943, **15**, 408.
[34] A. L. Chaney and H. J. Magnuson, *ibid.*, 1940, **12**, 691.
[35] *J. Assoc. Off. Agric. Chem.*, 1939, **22**, 121. [36] *Biochem. J.*, 1944, **38**, 195.
[37] *Ibid.*, 1943, **37**, 598. [38] *Ind. Eng. Chem. Anal.*, 1942, **14**, 82.
[39] *Ibid.*, p. 442. [40] *Analyst*, 1942, **67**, 279.

the latter being nearly all removed from the nitric acid solution by sulphuric acid, and the molybdenum-blue reagent applied to the filtrate.[41]

C. C. Cassil [42] recommends passing arsine into mercuric chloride solution, titrating this by adding excess of an iodine solution containing enough iodide to hold excess of mercury in solution, and back titrating with thiosulphate.

Various Methods.—Polarography is said [43] to be possible only in acid solution; the shape of the polarogram is influenced by the supporting electrolyte, and sometimes four waves are visible. K. Bambach [44] collects arsine in mercuric chloride solution, heats this to convert arsenides into arsenites, and at pH 6 precipitates mercury with hydroxylamine. The clear solution is polarographed after addition of hydrochloric acid. In 1·5N-acid the half-wave is at — 0·35 v., in 0·5N-acid at — 0·5 v. The process is more accurate than that of Gutzeit and quicker than colorimetry. J. J. Lingane [45] says that As^{V} is not reduced at the dropping-mercury cathode, but As^{III} is reduced in two steps; in N-hydrochloric acid the wave height at —0·8 v. is proportional to concentration. An ill-defined wave at — 0·9 v. may correspond to reduction to arsine. Tartrate or alkali suppresses the wave, and 0·1% of gelatin should be present.

S. Torrance [46] states that if a solution of copper containing less than one-fifth of its weight of arsenic in hydrochloric acid is electrolysed, all the arsenic is deposited with the copper. If the deposit is redissolved and electrolysed in sulphuric solution, only copper is deposited, leaving all the arsenic in solution.

Among noteworthy miscellaneous methods are the distillation of arsenious chloride from solutions containing tungsten,[47] the determination of arsenates and selenates in presence of one another,[48] a microtechnique for determination as magnesium ammonium arsenate,[49] the mechanism of Bettendorf's test,[50] and a method for quantitatively separating arsenic from copper by co-precipitation with hydrated manganese oxide.[51] In determining arsenic in sulphur, S. J. Fainberg and G. A. Taratorin [52] dissolve the latter in boiling sulphite solution, and add copper sulphate and acetic acid, whereupon all the arsenic is carried down as trisulphide by the copper sulphide; or the sulphur is dissolved in alkaline hydrogen peroxide, and the arsenic determined colorimetrically.

[41] E. A. Coakill, *Analyst*, 1938, **63**, 801.

[42] *J. Assoc. Off. Agric. Chem.*, 1938, **21**, 198; (with H. J. Wichmann) 1939, **22**, 436; 1941, **24**, 196.

[43] T. A. Krinkova, *Zavod. Lab.*, 1940, **9**, 950.

[44] *Ind. Eng. Chem. Anal.*, 1942, **14**, 265. [45] *Ibid.*, 1943, **15**, 583.

[46] *Analyst*, 1938, **63**, 104; 1939, **64**, 263.

[47] T. Millner and F. Künos, *Z. anal. Chem.*, 1936, **107**, 96.

[48] J. Milbauer, *ibid.*, 1937, **109**, 171.

[49] F. Hecht and M. von Mack, *Mikrochem. Acta*, 1937, **2**, 218.

[50] W. B. King and F. E. Brown, *J. Amer. Chem. Soc.*, 1939, **61**, 968.

[51] C. L. Luke, *Ind. Eng. Chem. Anal.*, 1943, **15**, 626.

[52] *Zavod. Lab.*, 1940, **9**, 1223.

Finally, an important paper [53] is devoted to analysis of large numbers of samples of foodstuffs or the like : after wet oxidation and removal of nitric acid, a few mg. of cadmium sulphate are added to the diluted solution and precipitated as sulphide, carrying down all the arsenic. The cadmium later serves as an internal standard. The sulphide is collected on a small suction filter dressed with powdered graphite, and after being dried is arced in a low-tension D.C. arc between graphite electrodes using a Hilger medium spectrograph to record the spectrum. Normally, visual comparison between the spectrogram and that of a standard sample suffices to show whether or not the specified limits for arsenic, etc., have been exceeded. The method is very expeditious and suitable for mass production methods of working, but as only 1 g. of sample is used, scrupulous care must be taken over " blanks " and absolute cleanliness of working.

H. N. W.

III. *Fluorine.*

Probably the determination of fluorine has changed more than that of any other element in recent years. H. H. Willard and O. B. Winter's procedure,[1] in which the fluorine was distilled as hydrosilicofluoric acid from an aqueous acid solution in which quartz powder was suspended, hydrolysed again to fluoride in the distillate, and titrated with N/100-thorium nitrate solution with alizarin-S as an indicator, was a remarkable advance in analytical technique. Much subsequent work has simply been refinement and amplification of this method.

Ashing.—The possibility of losing fluorine during ashing or calcining operations cannot be too greatly stressed. J. M. Sanchis [2] adds sodium hydroxide solution before ashing in platinum, and distilling at 135—140° with sulphuric acid ; chlorine interferes, especially in presence of manganese, and should be removed by means of sodium nitrite. Finally, the degree of fading caused by various aliquots of the distillate is compared against standards, using zirconyl alizarin-S lake as is now usual. In the micro-determination of fluorine in blood,[3] loss of fluorine is reduced by first charring in a porcelain crucible, then transferring to a gold one ! The ash must not be sintered. J. M. Kolthoff's colorimetric reagent [4] is recommended (*i.e.*, zirconyl chloride and purpurin solution). Wine can be ashed without loss of fluorine,[5] and one distillation at 135° with perchloric acid removes all the fluorine. For determining fluorine in impregnated wood, B. Ikert [6] adds chromium acetate and calcium acetate solutions before ashing, as this is stated to lead to a better recovery. In analysis of wool moth-proofed with fluorine compounds, F. F. Elsworth and J. Barritt [7] moisten the wool with N/2-sodium carbonate solution, and ignite below a

[53] D. A. Harper and N. A. Strafford, *J. Soc. Chem. Ind.*, 1942, **61**, 74.
[1] *Ind. Eng. Chem. Anal.*, 1933, **5**, 7. [2] *Ibid.*, 1934, **6**, 134.
[3] H. Wulle, *Z. physiol. Chem.*, 1939, **260**, 169.
[4] *Ind. Eng. Chem. Anal.*, 1934, **6**, 118.
[5] H. G. Rempel, *ibid.*, 1939, **11**, 378.
[6] *Chem.-Ztg.*, 1939, **63**, 754. [7] *Analyst*, 1943, **68**, 298.

red heat. For fluorine in coal (25—150 parts per million), H. E. Crossley [8] burns it with sodium carbonate at a low temperature, or ignites it in a calorimetric bomb. In the first case he follows combustion by a fusion, separates silica as usual, and distils. In the second case interference by nitrates is overcome by reduction with a zinc–copper couple before distillation. The distillates are compared visually with standards, the author correctly observing that the zirconium lake colours are not suitable for absolute colorimetry. Calcium or magnesium peroxide is suggested [9] as an adjunct in the ashing of soils. In the presence of much silica, magnesium peroxide leads to low recoveries, but in presence of much organic matter and low silica content, it is preferred to calcium peroxide, and leads to a less violent reaction. Magnesium acetate may be used as an ashing agent;[10] it is free from fluorine and gives very concordant results after ashing at 570°.

Distillation.—This has been very thoroughly studied. Recovery of fluorine decreases with increasing volume in the distilling flask, and the presence of non-volatile acids hinders the distillation; the effect of volume is less marked with sulphuric acid, volatilisation is slowest with phosphoric acid, and recovery is more rapid at higher temperatures.[11] D. S. Reynolds and his co-workers [12] describe a steam-distillation in which the temperature is regulated by blowing in steam rather than by dropping in water, and its application to phosphate rock. The process is typical; 0·5 g. is distilled with 15 ml. of 2 : 1 perchloric acid, the temperature being maintained at 125—150°. Recovery is complete when 150 ml. have been distilled. If pyrites or organic matter is present, distillation is preceded by oxidation with permanganate. In the presence of colloidal silica or alumina, which obstinately retain fluorine, it is necessary [13] to make a preliminary distillation with sulphuric acid at 165°.

Titration.—The use of thorium salts for this purpose has been thoroughly studied. The amount of indicator in Willard and Winter's titration [1] with N/100-thorium nitrate must be kept constant,[14] and changes in pH cause errors proportional to the change and to the fluorine content. The best pH is 2·5—3·0, and a back titration is preferred. By using a buffer of half-neutralised N-chloroacetic acid, pH 3, R. J. Rowley and H. V. Churchill [15] avoided the use of alcohol, and titrated with N/10-$Th(NO_3)_4$, extending the range considerably. R. A. Clifford [16] measured the colour during the titration, using a photometer, and showed that for very small quantities there is

[8] *J. Soc. Chem. Ind.*, 1944, **63**, 280.
[9] W. H. MacIntyre and J. W. Hammond, *J. Assoc. Off. Agric. Chem.*, 1939, **22**, 231.
[10] W. E. Crutchfield, jr., *Ind. Eng. Chem. Anal.*, 1942, **14**, 57.
[11] D. Dahle and H. J. Wichmann, *J. Assoc. Off. Agric. Chem.*, 1936, **19**, 303; 1937 **20**, 297.
[12] *Ibid.*, 1936, **19**, 156; *Ind. Eng. Chem. Anal.*, 1939, **11**, 21.
[13] D. Dahle and H. J. Wichmann, *J. Assoc. Off. Agric. Chem.*, 1936 ,**19**, 320.
[14] D. Dahle, R. W. Bonar, and H. J. Wichmann, *ibid.*, 1938, **21**, 459.
[15] *Ind. Eng. Chem. Anal.*, 1937, **9**, 551.
[16] R. A. Clifford, *J. Assoc. Off. Agric. Chem.*, 1940, **23**, 303.

no " end-point," but a gradual change. He recommends titration to an intermediate colour, adding the same amount of 0·0004M-$Th(NO_3)_4$ solution to a " blank " containing the same quantity of indicator, and back-titrating with a standard (1 ml. = 0·01 mg. F) solution until the colours match. The difficulties of titrating very small amounts are reviewed.[17] Fluorine concentrations of 2—50 μg. and 0·2—5 mg. per 10 ml. were studied at pH 3 in presence and in absence of chloroacetic acid, and the thorium nitrate was 0·0175N. In alcoholic solution in the lower range, fair agreement was obtained, but the buffered solution gave high results; in aqueous solutions all results were too high, the buffered solutions again being the higher. In the 0·2—5 mg. range all the systems gave good results. Micro-quantities of fluorine in aqueous solution must thus be titrated by empirically standardised solutions, but for alcoholic solutions the stoicheiometric factor can be used.

Colorimetric Methods.—Numerous methods exist for colorimetric estimation of small amounts of fluorine, apart from the " titration-colorimetry." [16,26,27] Except in waters, it is almost always necessary to isolate the fluorine by distillation. The development of the zirconium–alizarin lake method is shown by three papers. In waters,[18] the usual amounts of manganese, aluminium, iron, silicate, sodium chloride and sulphate have very small effect, but ferric iron and phosphate completely vitiate the procedure. The sample is made acid with sulphuric and hydrochloric acids, a solution of zirconyl nitrate + alizarin-S added, and the whole heated to boiling and allowed to stand overnight. It is compared in Nessler cylinders with standards similarly treated. W. L. Lamar and C. G. Seegmiller [19] describe a procedure in which only sulphuric acid is added to the water, which is not boiled, but allowed to stand overnight with the reagent, before comparison with standards containing 0·02—0·24 mg. of fluorine. What is perhaps the best method for fluorine in small amounts in waters, etc., is given by A. P. Black *et al.*[20] and by R. D. Scott.[21] The reagent contains alizarin-S and zirconium oxychloride, made 1·5N with regard to both hydrochloric and sulphuric acid, thus reducing to a minimum interference from chlorides and sulphates. The colour is bleached by fluoride ions, and the best range of standards is 0·01—0·1 mg. of fluorine per 100 ml., but it can be extended to 0·18 mg. Iron and aluminium both interfere if present to more than 0·5 parts per million. The reaction is complete in 2 hours, and the reagent keeps very well. N. A. Talvitie [22] suggests a thorium nitrate reagent buffered at 3·5, and containing 0·008% of alizarin-S. The sample is neutralised with dilute nitric acid before applying the reagent. The method is simple and rapid, and would detect 0·1 part per million on a 100-ml. sample.

Two papers utilise the bleaching of the iron complex with 7-iodo-

[17] J. W. Hammond and W. H. MacIntire, *J. Assoc. Off. Agric. Chem.*, 1940, **23**, 398.
[18] O. J. Walker and G. R. Finlay, *Canadian J. Res.*, 1940, **18**, 151.
[19] *Ind. Eng. Chem. Anal.*, 1941, **13**, 901.
[20] *J. Amer. Water Works Assoc.*, 1941, **33**, 1965. [21] *Ibid.*, 1941, **33**, 2018.
[22] *Ind. Eng. Chem. Anal.*, 1943, **15**, 620.

8-hydroxyquinoline-4-sulphonic acid ("ferron") by fluoride ions. For fluorine in rocks,[23] the sample is fused with alkali, and silica is separated by a modified Berzelius–Rose method, finally with zinc oxide and ammonium carbonate. An aliquot of the filtrate is taken, and a standard prepared containing the same concentration of salts. Each is treated with 2 ml. of the ferric "ferron" reagent, and the standard titrated with a N/50-sodium fluoride solution until the colours match; a difference of 0·05 mg. of fluorine is readily perceptible, and the range is 0·1—1·5 mg. Alternatively,[24] the fluorine may be isolated by a preliminary distillation at 165° with sulphuric acid, the distillate being neutralised, concentrated, and redistilled at 135° with perchloric acid. An aliquot containing < 5 mg. of fluorine is treated with the reagent, and its extinction measured with a photometer, using a red filter. The fluorine content is read from a graph. In the peroxytitanic sulphate method [25] aluminium ions largely counteract the bleaching effect of fluorine on the colour; if the same amount of pertitanate solution is added to each of two aliquots, and aluminium to one of them, provided the pH be not changed, the difference in colour will be proportional to the fluorine content, irrespective of the colour of the original solution.

There are two important papers on fluorine in foods.[26,27] The two methods are similar; in the English method on a 1-g. sample, 1 part per million can be determined with an accuracy of 0·15 part; in the American, the best amount of fluorine to have present is 30—70 μg. The originals must be consulted for details, but reagents must be prepared especially to be free from fluorine, and a little ($< 1·5$ μg.) always comes from the glass. Thorium nitrate solution (0·025%) is added to the final distillate to a faint pink colour, the same volume added to the "blank," and then standard sodium fluoride solution (1 ml. = 0·01 mg. F) to the blank until the colours match. Further applications are recorded in various papers.[28]

Miscellaneous.—The lead chlorofluoride method is applied to insecticides.[29] For the determination of fluoride in organic compounds two procedures are given. A new technique is described by P. J. Elving and W. B. Ligett.[30] The compound is heated in a closed tube, like a Carius tube, with metallic potassium cut into small pieces, air having been displaced by adding a few ml. of ether, which is sucked off as vapour, removing also water. After exhaustion, the tube is sealed, and heated to 400°. After cooling, excess of potassium is destroyed by ethanol, the residue dissolved, the solution filtered, and fluorine determined, *e.g.*, as lead chlorofluoride. M. L. Nichols and J. S. Olsen [31] commence by fusion with sodium peroxide, potassium carbonate, and sugar in a Parr bomb; after extraction and neutralisation, fluorine is titrated potentiometrically with cerous nitrate.

[23] J. J. Fahey, *Ind. Eng. Chem. Anal.*, 1939, **11**, 362.
[24] P. Urech, *Helv. Chim. Acta*, 1942, **25**, 1115.
[25] D. Dahle, *J. Assoc. Off. Agric. Chem.*, 1937, **20**, 505.
[26] Society of Public Analysts Sub-Committee, *Analyst*, 1944, **69**, 243.
[27] Anon., *J. Assoc. Off. Agric. Chem.*, 1944, **27**, 90.
[28] P. A. Clifford, *ibid.*, p. 246; Anon., *ibid.*, p. 98.
[29] Anon., *ibid.*, p. 75.
[30] *Ind. Eng. Chem. Anal.*, 1942, **14**, 449.
[31] *Ibid.*, 1943, **15**, 342.

The concentration of fluorine in air is determined automatically by aspirating it through a solution containing ferric chloride, potassium thiocyanate, and persulphate. Fluorine bleaches the red colour, and the change is measured photoelectrically.[32] Full details of apparatus and procedure for sampling anhydrous hydrogen fluoride are given.[33, 34] It is then diluted by addition to ice in a special weighing vessel, after which the solution is analysed for hydrofluoric, hydrosilicofluoric, sulphurous and sulphuric acids.

Qualitative Tests.—J. Fischer [35] adds to the neutral fluoride solution eosin, lanthanium acetate, and sodium acetate; on boiling, cooling, and centrifuging, a red precipitate indicates fluorine; 2 μg. can be detected. E. R. Caley and J. M. Ferrer [36] describe apparatus for a micro-etching test, and suggest the preparation of a series of standard etched microscope coverglasses, the amounts dealt with ranging down to 0·05 mg. of calcium fluoride.

H. N. W.

IV. *Organic Microchemical Analysis.*

In 1942 an excellent review of the recent literature was published,[1] and the following is a brief note on progress since then. The determination of specific compounds is not reported, but only analysis for elements or radicals, with notes on new apparatus.

The American Chemical Society recommendations for apparatus for the determination of sulphur and the halogens (Pregl's methods) have been published,[2] and G. H. Wyatt [3] reviews all kinds of micro-volumetric apparatus. The error of a single weighing on a microbalance is ± 3 μg., most of which is due to the placing of the rider, but results are stated [4] to be described as surprisingly poor,[5] the standard deviation being 3·4 μg., and the largest error to be expected from a single weighing being 7 μg. A long article [6] is devoted to the errors of a Kuhlmann balance; the tracing of errors is described, and every possible error due to environment, construction, wear, design, etc., is considered. Random errors amount to about 5 μg., and suggestions are made to reduce this to 1 μg. Simple apparatus are described for micro-ammonia distillation [7] and for semimicro-alkoxyl determinations,[8] preferably by F. Vieböck and C. Brechner's method.[9] The latter apparatus can also be used for wet methods of halogen determination. A V-shaped micro-pyknometer made of capillary tubing, and with a capacity of 0·01—0·02

[32] L. S. Tschemodanova, *Zavod. Lab.*, 1939, **8**, 1248.
[33] U.S.A. Manufacturing Chemists' Association, *Ind. Eng. Chem. Anal.*, 1944, **16**, 483.
[34] C. F. Swinehart and H. F. Flisik, *ibid.*, p. 419.
[35] *Z. anal. Chem.*, 1936, **104**, 344. [36] *Microchim. Acta*, 1937, **1**, 160.
[1] L. T. Hallett, *Ind. Eng. Chem. Anal.*, 1942, **14**, 956.
[2] G. L. Royer, H. K. Alber, L. T. Hallett, and J. A. Kuck, *ibid.*, 1943, **15**, 230.
[3] *Analyst*, 1944, **69**, 81.
[4] M. Corner and H. Hunter, *ibid.*, 1941, **66**, 149.
[5] Committee on Micro-balances, American Chem. Soc., *Ind. Eng. Chem. Anal.*, 1943, **15**, 415.
[6] A. H. Corwin, *ibid.*, 1944, **16**, 258. [7] R. Markham, *Biochem. J.*, 1942, **36**, 790
[8] T. White, *Analyst*, 1943, **68**, 366. [9] *Ber.*, 1930, **63**, 3207.

ml., is described;[10] it can be mounted on a board carrying 2 scales, and calibrated with the liquid at various levels, read on the scales, and a graph plotted to show volumes at various levels in the arms. Results are quoted correct to 3 or 4 digits. Methoxyl and ethoxyl groups may be determined in the same molecules;[11] the two alkyl iodides are collected in a 10% solution of trimethylamine in alcohol, three receivers in series being used. After 50 minutes the receivers are washed out with alcohol and water, the solution evaporated to dryness, and trimethylethylammonium iodide extracted with a saturated solution of tetramethylammonium iodide in absolute alcohol. The tetramethylammonium iodide thus remaining is oxidised to iodic acid, and determined as usual. Results quoted for samples of 10—15 mg. are good. An improved semimicro-method for alkoxy-groups in cellulose ethers, etc., is given.[12] A. A. Houghton[13] describes a modified apparatus for simple and rapid working of Viebock's method;[9] the apparatus requires very little attention and is suitable for repetition work. An analysis is completed in less than an hour, and errors on 10-mg. samples are insignificant.

Hydroxyl groups are determined[14] by weighing 2—10 mg. into a melting-point tube sealed at one end, followed by a weighed amount (20—25 mg.) of acetic anhydride, and excess (not weighed) of pure pyridine. The tube is sealed, centrifuged to mix the reagents, and allowed to stand for 24 hours. It is broken under water, and excess of acetic anhydride titrated with 0·04N-sodium hydroxide. A blank is run simultaneously.

Several papers treat of halogens. E. W. Peel, R. H. Clark, and E. C. Wagner[15] fuse 15—20 mg. of substance in a Parr micro-bomb, with sodium peroxide; they prefer gravimetric procedures, and weigh liquids in gelatin capsules rather than capillary tubes. A. F. Colson[16] describes a modified apparatus for volatile compounds, for which the Parr bomb is unsuitable. In a modified Pregl's apparatus, the compound is volatilised in a stream of air or oxygen, the stream passing through a heated platinum cylinder to act as a catalyst. In the cylinder is a glass rod, which forces the gas stream through the small annulus between the rod and the cylinder, thus ensuring efficient contact. The chlorine is absorbed in solid sodium carbonate and granular lime, the mixture dissolved in dilute nitric acid, reduced with hydrazine, and the chloride finally weighed as silver salt. Excellent results are quoted. P. J. Hardwick[17] determines bromine in biological fluids by heating in a sealed tube with sodium ethoxide solution, evaporating the product, and after gentle calcination, dissolving the residue, neutralising it, and oxidising it either (i) to bromate with hypochlorite or (ii) to bromine with chloramine-T

[10] A. A. Houghton, *Analyst*, 1944, **69**, 346.
[11] L. M. Cooke and H. Hibbert, *Ind. Eng. Chem. Anal.*, 1943, **15**, 24.
[12] E. P. Samsel and J. A. McHard, *ibid.*, 1942, **14**, 750.
[13] *Analyst*, 1944, **69**, 363.
[14] J. W. Peterson, K. W. Hedberg, and B. E. Christensen, *Ind. Eng. Chem. Anal.*, 1943, **15**, 225.
[15] *Ibid.*, 1943, **15**, 149. [16] *Analyst*, 1942, **67**, 47. [17] *Ibid.*, p. 223.

in presence of fluorescein, the red colour (eosin) produced being a measure of bromine present.

G. Ingram [18] deals with the uses of mercuric oxycyanide in micro-volumetric analysis. In neutral solution, this reagent reacts with halogen salts to liberate an equivalent quantity of hydroxyl, to be titrated with N/100-sulphuric acid. He describes applications to the determination of halogens, a special apparatus for the combustion being described. By using oxygen saturated with water, the formation of sulphur trioxide mist from sulphur compounds is avoided. If both sulphur and halogens are present, total acid formed may be titrated as usual, then halogen by means of the oxycyanide. The reagent may also be applied to the determination of alkoxyl groups.

W. B. Price and L. Woods [19] describe a technique for the analysis of minute bubbles of gases, *e.g.*, from the bubbles in glass. The bubble is measured under a slide in glycerol by means of a microscope, and after treatment with reagents, measured again. The smallest bubble can be 0·2 mm. in diameter or 0·004 mm. in volume, and hydrogen, oxygen, hydrogen sulphide, carbon monoxide and dioxide can be determined.

A semimicro-apparatus is described [20] for determining Reichert, Polenske, and Kirschner values in fats. The formyl group may be determined [21] similarly to acetyl, except that the formic acid produced may be either titrated with alkali or oxidised by bromine in N/100-solution, thus making the determination specific. The semimicro-determination of esters, which are heated to 60° in closed 25-ml. flasks with 2N-sodium hydroxide in 90% methanol, is discussed with reference to steric hindrance.[22]

R. Belcher and C. E. Spooner [23] deal with attempts to hasten the usual Liebig or Pregl form of analysis by using rapid air rates, and to simplify the apparatus by use of empty tubes. Originally put forward as a macro-method for the ultimate analysis of coal, the method embodies novel features. The coal sample is gradually introduced into a tube heated to 1350°, and no packing is used except a silver spiral at the cool exit end to absorb halogens and sulphur. The oxygen rate is 300 ml./minute, and a complete combustion occupies ten minutes. Carbon, hydrogen, sulphur, and chlorine can be determined simultaneously. Micro- and semimicro-procedures are also given. Combustion is conducted in an empty silica tube, a roll of silver gauze serving to trap halogens and sulphur; oxides of nitrogen are trapped by N/50-potassium permanganate or dichromate in sulphuric acid. The oxygen rate is 50 ml./minute. Carbon, hydrogen, and sulphur can be determined simultaneously, the last being dissolved off the silver spiral as silver sulphate, after which the spiral is again weighed. It is claimed these methods are more rapid and simpler than the standard procedure. The last

[18] *Analyst.*, 1944, **69**, 265. [19] *Ibid.*, p. 117.
[20] B. Dyer, G. Taylor, and J. H. Hamence, *ibid.*, 1941, **66**, 355.
[21] J. F. Alicano, *Ind. Eng. Chem. Anal.*, 1943, **15**, 704.
[22] J. Mitchell, D. M. Smith, and F. S. Money, *ibid.*, 1944, **16**, 410.
[23] *Fuel*, 1941, **20**, 130; *Ind. Chem.*, 1943, **19**, 653; *J.*, 1943, 313; R. Belcher, *J. Inst. Fuel*, 1944, **17**, 160.

paper was followed by an interesting discussion,[24] the opinion being that good results were obtained by the macro- and the semimicro-procedure, but that on the micro-scale, trouble was caused by formation of oxides of nitrogen.

G. Ingram [25] describes a new method for determining carbon and hydrogen. In a silica combustion tube the packing is placed in boats. The catalyst is a special mixture of ceria, litharge, silver dichromate, and silver oxide, and also a roll of copper gauze filled with a mixture of ceric and vanadic oxides. The combustion is conducted at 500°, and is complete in 50 minutes. Silver vanadate on pumice as the main oxidation filling is superior to copper oxide and lead chromate, or to platinum contacts.

A. E. H.
H. N. W.

A. E. Heron.
H. N. Wilson.

[24] *J. Inst. Fuel*, 1944, **18**, Suppl. p. 51.
[25] *J. Soc. Chem. Ind.*, 1942, **61**, 112; 1943, **62**, 175.