

BIOCHEMISTRY.

1. INTRODUCTION.

IN the course of comparatively a few years biochemistry has so developed that, from being a little-explored side-line of chemistry or physiology, it has become itself a vast new tract for investigation, with numerous sub-divisions of its own, each of which in turn forms the subject for intensive and specialised study by teams of researchers. Indeed so specialised have the various branches become that it is felt that the time is ripe to make a new departure with this issue of the *Annual Reports*, and to call in the aid of experts to deal with recent developments in their own lines of work.

Nutrition still accounts for by far the largest output of papers—no fewer than 5,127 abstracts are included in the year's list in *Nutrition Abstracts and Reviews*—and, following the custom of these Reports for a good many years past, it is accordingly placed first in order of attention. The increased importance of this subject in time of war needs no emphasis. Next follows an account, by J. R. Marrack, of some of the more remarkable of the recent findings in another field of equally great practical importance, namely immuno-chemistry. Still considering “the animal as a whole,” we turn then to a discussion of the chemical control of the body through the agency of the secretions of the ductless glands. The number of such hormones already known exceeds that of the vitamins, and so with the restricted space at his disposal E. Kodicek has wisely limited his attention to one gland only, or a portion of it, namely, the anterior pituitary. Turning now, in a sense, from the needs of the whole animal to the chemistry of single components of its tissues, A. Neuberger deals with some significant recent advances in the study of nitrogenous substances, and J. F. Danielli reviews certain underlying physicochemical principles of concern to the biochemist. The enzymes are substances of such universal significance for all living tissues that an adequate understanding of their nature and their mode of action is a prime necessity for the advance of all aspects of biochemical knowledge; this field is covered by D. J. Bell in the section on biochemical catalysis. Leaving the animal kingdom behind, F. W. Norris, as in several recent years, contributes a section dealing in a general way with the chemical changes which occur in the living plant. A special aspect of this, glucoside formation, is then touched by R. Hill. Finally, coming to micro-organisms,

(Miss) M. Stephenson and E. F. Gale devote their section on bacterial biochemistry to two topics which have been much to the fore in recent literature, namely, "accessory food factors" and nitrogen metabolism.

L. J. H.

2. NUTRITION AND VITAMINS.

Vitamin A.

Human Requirements.—Interest has centred largely on the detection of deficiency by the dark-adaptation test¹ and the measurement of the daily human requirement for the vitamin. Estimates of the latter seem to work out in reasonably good agreement. In one particularly convincing experiment² ten volunteers were deprived of the vitamin for 188 days, until their visual sensitivity was 1/28 of the normal, and it was then ascertained that 2000—2500 I.U. of vitamin A, or 5000 I.U. of carotene, daily were the smallest amounts which would bring about a *slow* improvement in adaptation. No vitamin A was present in the blood until the dose of 2500 I.U. of vitamin A was reached or exceeded. The foregoing estimate errs perhaps in being on the low side, since there seems no doubt that larger intakes would be needed for a more prompt cure. L. E. Booher and E. C. Callison³ found the requirement to be about 4000 and 7000 I.U. of carotene, given as cooked peas and cooked spinach respectively (recalculated to 70 kg. of body-weight); and W. v. Drigalski⁴ considers that about 9800 I.U. of carotene daily are needed, increasing to about double or treble in lactation or late pregnancy.⁵ H. R. Guilbert *et al.*,⁶ after reviewing past data and comparing different mammalian species, cite 1400—4200 I.U. for vitamin A and 2800—14,000 I.U. for carotene, the former figure in each instance being the *minimum* for freedom from clinical symptoms, and the latter the *optimum* for normal adaptation, growth, and significant storage. Guilbert points out that the requirement for vitamin A remains relatively constant for a surprisingly large number of species, including the ox, sheep, pig, horse, and rat—namely, about 25 I.U. per kg. of body-weight for vitamin A, and about 50 I.U. for carotene. The differences between the vitamin and the "pro-vitamin" are of course due to the less complete absorption or assimilation of the latter, which may also

¹ Compare *Ann. Reports*, 1939, **36**, 338.

² K. H. Wagner, *Z. physiol. Chem.*, 1940, **264**, 153.

³ *J. Nutrition*, 1939, **18**, 459.

⁴ *Klin. Woch.*, 1939, **18**, 1269.

⁵ W. v. Drigalski and H. Kung, *ibid.*, p. 1318.

⁶ H. R. Guilbert, C. E. Howell, and G. H. Hart, *J. Nutrition*, 1940, **19**, 91.

vary considerably according to the digestibility or the method used for cooking the vegetable containing the carotene.

Conditioned Deficiency in Man.—Several workers have confirmed that deficiency of vitamin A, as detected by the dark adaptation test, is commonly found in diseases of the liver (*e.g.*, cirrhosis, parenchymatous liver disease).^{7, 8, 9} The deficiency runs parallel with the degree of damage of the liver,⁸ and may be due either to inadequate absorption (as in obstructive jaundice and steatorrhœa), or to deficient storage caused by the hepatic dysfunction, and sometimes also to a lowered intake.⁹ In juvenile diabetes dysadaptation is common, and the explanation is thought to be an inability to convert carotene into vitamin A.¹⁰ Further indications have been given also of a frequent correlation between renal calculus and deficiency of vitamin A.¹¹

Incidence of Deficiency.—Various investigators agree that dark adaptation tests give a reliable index of "partial deficiency," provided that the method is used with the proper precautions. G. Sankaran,¹² examining 600 convicts in a jail in Calcutta, found that 43% of the lower-class groups and 17% of the better-class groups showed a *high* degree of deficiency. This may be compared with the results of L. J. Harris and M. A. Abbasy,¹³ who found a comparatively *mild* degree of deficiency present in about 30% of poor working-class children in England.

Tests for Deficiency in Man.—O. D. Abbott, C. F. Ahmann, and M. R. Overstreet¹⁴ in U.S.A. and K. H. Wagner² in Germany point out that a differential leucocyte count is of value in diagnosing deficiency. Wagner found that after 188 days on a deficient diet the thrombocytes, too, were 70—85% below normal, and there was a leucopenia with a "right shift" of the differential count. Thus, four distinct criteria are now available for assessing deficiency in man: (1) dark adaptation, (2) the level of vitamin A in the blood, (3) presence of cornified cells in scrapings from the bulbar conjunctiva, (4) blood counts.

Experimental Avitaminosis A.—H. Mellanby¹⁵ has recorded defects in the dental structure of young rats when their mothers'

⁷ A. J. Patek and C. Haig, *J. Clin. Invest.*, 1939, **18**, 609.

⁸ M. G. Wohl and J. B. Feldman, *J. Lab. Clin. Med.*, 1940, **25**, 485.

⁹ M. Salah, *J. Egypt. Med. Assoc.*, 1940, **23**, 153.

¹⁰ J. G. Brazer and A. C. Curtis, *Arch. intern. Med.*, 1940, **65**, 90; cf. also *J. Clin. Invest.*, 1939, **18**, 495.

¹¹ H. Long and L. N. Pyrah, *Brit. J. Urol.*, 1939, 216.

¹² Ann. Report All-India Inst. Hygiene and Pub. Hlth., Calcutta, 1939, p. 33.

¹³ *Lancet*, 1939, ii, 1299, 1355.

¹⁴ *Amer. J. Physiol.*, 1939, **126**, 254.

¹⁵ *Brit. Dental J.*, 1939, **67**, 187.

diets were lacking in vitamin A. These include degenerated enamel organs, poorly calcified dentine, ossifying cells in pulp, abnormalities in the mandible. According to M. E. Sauer,¹⁶ the epithelial metaplasia of avitaminosis A is independent of any nerve change. C. A. Baumann and T. Moore¹⁷ have been unable to confirm the theory of a specific antagonism between thyroxin and vitamin A.

Workers in dietetics will have noted with interest that the so-called "light-white casein," frequently used in nutritional experiments, is contaminated with appreciable amounts of vitamin A;¹⁸ also that the disproportionately high values suggested for cheese have not been confirmed.¹⁹ Reference must be made to a valuable review²⁰ covering both chemical and physiological aspects of carotene and related pigments.

Vitamin B₁.

Vitamin B₁ and the Pyruvate Oxidation System.—It is becoming clear that the system by which pyruvic acid is oxidised, under the influence of vitamin B₁ (as its pyrophosphate ester, cocarboxylase), may vary in some of its details from one tissue to another. In brain, or kidney cortex, C₄ dicarboxylates (*e.g.*, succinate and fumarate) seem to be of importance, and other components of the system include inorganic phosphate, Mg⁺⁺ (or Mn⁺⁺), adenine nucleotide and probably cozymase (pyridine nucleotide).²¹ In pigeon breast muscle²² or liver,²³ on the other hand, the reaction appears to proceed through the citric acid cycle—*i.e.*, citric acid is formed as an intermediate—and among the principal products formed may be acetoacetate and α-ketoglutarate. The last-mentioned substance has been identified in large amounts in the urine of deficient rats,²⁴ and the quantity of citric acid is said to rise steeply shortly after a cure.²⁵

An interesting development has followed the discovery of H. G. Wood and C. H. Werkman²⁶ that "propionic-acid bacteria" absorb carbon dioxide. They suggested that the mechanism might involve the addition of carbon dioxide to pyruvate to give oxaloacetate. H. A. Krebs and L. V. Eggleston²⁷ have now propounded the

¹⁶ *Anat. Rec.*, 1939, **74**, 223.

¹⁷ *Biochem. J.*, 1939, **33**, 1639.

¹⁸ M. K. Maitra and T. Moore, *ibid.*, p. 1648.

¹⁹ A. W. Davies and T. Moore, *ibid.*, p. 1645.

²⁰ R. A. Morton, *Chem. and Ind.*, 1940, **59**, 301.

²¹ I. Banga, S. Ochoa, and R. A. Peters, *Biochem. J.*, 1939, **33**, 1980.

²² H. A. Krebs and L. V. Eggleston, *ibid.*, 1940, **34**, 442.

²³ E. A. Evans, *ibid.*, p. 829.

²⁴ P. E. Simola, *Biochem. Z.*, 1939, **302**, 84.

²⁵ H. A. Sober, M. A. Lipton, and C. A. Elvehjem, *J. Biol. Chem.*, 1940, **134**, 605.

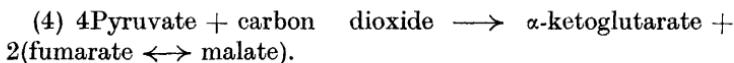
²⁶ *Biochem. J.*, 1938, **32**, 1262; 1940, **34**, 7, 129.

²⁷ *Ibid.*, p. 1383.

hypothesis that in certain animal tissues, e.g., in liver, and in bacteria, the vitamin is concerned not in the direct oxidation of pyruvate as hitherto supposed but in a preliminary carboxylation, analogous with the decarboxylation known to occur in yeast. This carboxylation results in the formation of oxaloacetate, and the latter then passes through the citric acid cycle, as follows :

- (1) Carbon dioxide + pyruvate \longrightarrow oxaloacetate;
- (2) 2Oxaloacetate + pyruvate \longrightarrow citrate + (fumarate \leftrightarrow malate) + carbon dioxide;
- (3) Citrate + oxaloacetate \longrightarrow α -ketoglutarate + (fumarate \leftrightarrow malate) + carbon dioxide;

the net result being the production of α -ketoglutarate plus fumarate in equilibrium with oxalate, according to the following equation :



However, this scheme does not appear to be of universal applicability; for example, these reactions do not occur in brain.

There can be no doubt that the interconnections between pyruvate oxidation and other related systems are most complicated. For example, P. J. G. Mann and J. H. Quastel²⁸ have shown that the production of acetylcholine from pyruvate is increased when vitamin B₁ is added to an aerobic preparation of polyneuritic brain. A further example of the complexity of the system is the finding that the oxidation of acetate by bacteria requires the participation of the vitamin.^{28a} Again, the observation²⁹ that insulin, like vitamin B₁, is able to lower the level of pyruvate in normal human blood suggests that the glycogen-glucose equilibrium and glucosemonophosphate formation are connected, if only indirectly, with the conversion of pyruvic acid.

Vitamin B₁ and Phosphorylating Mechanisms.—It is now clearly established that a cycle of phosphorylation accompanies the enzymic oxidation of pyruvate. In a preparation of brain, the rôle of adenylic acid seems to be to carry phosphate to hexosemonophosphate to form hexosediphosphate.³⁰ Phosphoglyceric and phosphopyruvic acids may have similar functions.²¹ F. Lipmann's experiments³¹ with *B. delbrückii* suggest that the unstable acetyl phosphate may be an intermediate phosphorylated derivative in the oxidation of pyruvic acid. As is well known, vitamin B₁ itself becomes phosphorylated in

²⁸ *Nature*, 1940, **145**, 856.

^{28a} J. H. Quastel and D. M. Webley, *ibid.*, 1939, **144**, 633.

²⁹ H. v. Euler and B. Höglberg, *Naturwiss.*, 1940, **28**, 29.

³⁰ S. Ochoa, *Nature*, 1940, **145**, 747; cf. also **146**, 267.

³¹ *J. Biol. Chem.*, 1940, **134**, 463.

the organism to form the biologically active cocarboxylase, and according to H. Weil-Malherbe³² it does so through the agency of adenine pyrophosphate; phosphoglyceric acid³³ also may accelerate the action.

Activation of Cocarboxylase by Free Vitamin B₁.—Some two years ago S. Ochoa³⁴ made the surprising observation that cocarboxylase activity is stimulated by the presence of free vitamin B₁. An elegant piece of work by H. G. K. Westenbrink and D. A. van Dorp³⁵ has now proved that the aneurin does this by inhibiting the hydrolysis of cocarboxylase by the phosphatases present in the tissue juices—*i.e.*, as a product of the reaction, it slows the net rate of the reaction.

Assessment of the Level of Nutrition.—The suggestion was made by G. G. Banerji and L. J. Harris³⁶ that the diminished tolerance towards the intermediates of carbohydrate metabolism observed in vitamin B₁ deficiency could be used as a test for assessing the level of nutrition. In rats the urinary excretion of pyruvate, or its level in the blood, is proportional to the degree of the deficiency; and this finding has been confirmed by other workers.³⁷ For detecting partial deficiencies in man, the procedure is slightly less simple, since it involves loading with test doses of glucose or pyruvate or other products of intermediate metabolism, followed by examination for the accumulation of abnormal amounts of such metabolites as pyruvic acid or α-ketoglutaric acid: the precise experimental details are still in process of being worked out.^{36, 38} An alternative technique is the direct determination of the vitamin in urine,³⁹ and this method has been carefully standardised by Y. L. Wang and J. Yudkin,⁴⁰ who have also calculated from their data the probable human requirements. Alternatively, vitamin B₁, or cocarboxylase, can be measured in the blood.^{41, 42}

³² *Biochem. J.*, 1939, **33**, 1997.

³³ M. A. Lipton and C. A. Elvehjem, *Nature*, 1940, **145**, 226.

³⁴ *Ibid.*, 1938, **141**, 831; see also S. Ochoa and R. A. Peters, *Biochem. J.*, 1938, **32**, 1501.

³⁵ *Nature*, 1940, **145**, 465.

³⁶ *Chem. and Ind.*, 1938, **57**, 1190; *Biochem. J.*, 1939, **33**, 1346.

³⁷ M. Shils, H. G. Day, and E. V. McCollum, *Science*, 1940, **91**, 341; H. v. Euler and B. Höglberg, *Naturwiss.*, 1939, **27**, 769.

³⁸ G. G. Banerji, *Biochem. J.*, 1940, **34**, 1329; see pp. 1332—1333; K. O. Elsom, F. D. W. Lukens, E. H. Montgomery, and L. Jonas, *J. Clin. Invest.*, 1940, **19**, 153; W. D. Robinson, D. Melnick, and H. Field, *ibid.*, p. 483.

³⁹ L. J. Harris and P. C. Leong, *Lancet*, 1936, i, 886; L. J. Harris, P. C. Leong, and C. C. Ungley, *ibid.*, 1938, i, 539; Y. L. Wang and L. J. Harris, *Biochem. J.*, 1939, **33**, 1356.

⁴⁰ *Ibid.*, 1940, **34**, 343.

⁴¹ R. Goodhart and H. M. Sinclair, *J. Biol. Chem.*, 1940, **132**, 11.

⁴² R. Goodhart, *ibid.*, 1940, **135**, 77.

Methods of Assay.—The bradycardia method⁴³ has been used for large-scale surveys of foodstuffs, in the East Indies, China, and elsewhere, and found to agree with various other methods (rat growth, rice-bird, colorimetric-diazo, etc.).⁴⁴ R. Goodhart⁴² has recorded modifications in Sinclair and Goodhart's method for cocarboxylase in blood, H. M. Sinclair⁴⁵ in the Schopfer-Meiklejohn "fungus" test for B₁ in blood, and H. G. K. Westenbrink⁴⁶ in the Ochoa-Peters reaction for B₁ and cocarboxylase in yeast.

Vitamin-B₂ Complex.

Nicotinic Acid (Pellagra-preventive Factor).—E. Kodicek⁴⁷ has made a valuable study of the method for estimating nicotinic acid with *p*-aminoacetophenone.^{47a} Both he and W. R. Aykroyd and M. Swaminathan⁴⁸ have compared the distribution of the vitamin in various cereals and draw attention to the rather surprising fact that rice and millet may have little or no greater activity than maize, notwithstanding the well-known association of the latter with pellagra. In keeping with the known physiological function of nicotinic acid as a constituent of the pyridine coenzymes (phosphopyridine nucleotides), it has been proved that a deficiency, in either dogs or pigs, causes a fall in the amount of coenzyme I in their livers and muscles.⁴⁹ The so-called Factor V (needed for cultivation of *Hæmophilus parainfluenzae*, and served by either the di- or tri-phosphopyridine nucleotides) is similarly said to be lowered in the livers and muscles—but not in other tissues—of deficient dogs.⁵⁰ Human erythrocytes are able to synthesise Factor V *in vitro* when incubated with the vitamin, or *in vivo* when the latter is ingested by mouth.⁵¹ The biochemical rôle of the pyridine and other coenzymes has been much studied (see, for example, M. Dixon and L. G. Zerfas⁵²), and is discussed more fully elsewhere in this Report.

⁴³ T. W. Birch and L. J. Harris, *Biochem. J.*, 1934, **28**, 602; cf. *Ann. Reports*, 1939, **36**, 340.

⁴⁴ S. J. E. Pannekoek-Westenburg and A. G. van Veen, *Geneesk. Tijdschr. Nederl.-Indië*, 1939, **79**, 2891; E. F. Yang and B. S. Platt, *Chinese J. Physiol.*, 1939, **14**, 259; *A.*, 1940, III, 233; cf. also D. G. H. MacDonald and E. W. McHenry, *Amer. J. Physiol.*, 1940, **128**, 608.

⁴⁵ *Biochem. J.*, 1939, **33**, 2027.

⁴⁶ *Enzymologia*, 1940, **8**, 97.

⁴⁷ *Biochem. J.*, 1940, **34**, 712, 724.

^{47a} L. J. Harris and W. D. Raymond, *ibid.*, 1939, **33**, 2037.

⁴⁸ *Indian J. Med. Res.*, 1940, **27**, 667.

⁴⁹ A. E. Axelrod, R. J. Madden, and C. A. Elvehjem, *J. Biol. Chem.*, 1939, **131**, 85.

⁵⁰ H. I. Kohn, J. R. Klein, and W. J. Dann, *Biochem. J.*, 1939, **33**, 1432; M. Pittman and H. F. Fraser, *Publ. Health Reps., Wash.*, 1940, **55**, 915.

⁵¹ H. I. Kohn and J. R. Klein, *J. Biol. Chem.*, 1940, **135**, 685.

⁵² *Biochem. J.*, 1940, **34**, 371.

(see p. 415) and in a recent review by J. H. Quastel.^{52a} Of clinical importance is the suggested use of nicotinic acid for treatment of Vincent's disease (trench mouth),⁵³ in sulphanilamide poisoning,⁵⁴ and in the so-called "encephalopathic syndrome."⁵⁵

Riboflavin.—Deficiency of riboflavin in man is marked not only by lesions of the lips and seborrhœic accumulations on the face,⁵⁶ but by ocular manifestations, particularly photophobia and keratitis. These corneal lesions can be cured or made to reappear at will by administering or withholding riboflavin.⁵⁷ Similarly in rats, in addition to the skin lesions,⁵⁸ ocular symptoms are prominent: in order of onset these comprise conjunctivitis, blepharitis, corneal opacity, vascularisation and ulceration of the cornea, and finally cataract. All except the cataract are cured by riboflavin without any other treatment.⁵⁹ Flavin is important, likewise, in the rearing of pigs⁶⁰ and poultry.⁶¹ A method for checking the biological activity of various flavins, using lactic acid bacteria, has been described.⁶²

Pantothenic Acid (Chick-pellagra Factor, Filtrate Factor).—The structure of pantothenic acid has now been settled.⁶³ The synthetic dextrorotatory compound^{64, 65, 66} has been tested on micro-organisms, on chicks and on rats and found to have the identical activity of the naturally occurring substance:^{65, 67} the racemic acid has 50% of the

^{52a} Lecture delivered before the Institute of Chemistry of Great Britain and Ireland, 4th October, 1940.

⁵³ J. D. King, *Lancet*, 1940, **2**, 32.

⁵⁴ J. F. Doughty, *J. Amer. Med. Assoc.*, 1940, **114**, 756; G. B. Cottini, *Dermatologica*, 1940, **81**, 83.

⁵⁵ N. Jolliffe, *Res. Publn. Ass. nerv. ment. Dis.*, 1939, **19**, 148; N. Jolliffe, K. M. Bowman, L. A. Rosenblum, and H. D. Fein, *J. Amer. Med. Assoc.*, 1940, **114**, 307.

⁵⁶ W. H. Sebrell and R. E. Butler, *Publ. Health Reps., Wash.*, 1939, **54**, 2121; N. Jolliffe, H. D. Fein, and L. A. Rosenblum, *New Engl. J. Med.*, 1939, **221**, 921.

⁵⁷ H. D. Kruse, V. P. Sydenstricker, W. H. Sebrell, and H. M. Cleckley, *Publ. Health Reps., Wash.*, 1940, **55**, 157.

⁵⁸ H. Chick, T. F. Macrae, and A. N. Worden, *Biochem. J.*, 1940, **34**, 580.

⁵⁹ M. M. El-Sadr, *Chem. and Ind.*, 1939, **58**, 1020.

⁶⁰ E. H. Hughes, *J. Nutrition*, 1940, **20**, 233.

⁶¹ A. E. Schumacher and C. F. Heuser, *Poultry Sci.*, 1939, **18**, 369.

⁶² E. E. Snell and F. M. Strong, *Enzymologia*, 1939, **6**, 186; R. E. Feeney and F. M. Strong, *J. Biol. Chem.*, 1940, **133**, proc. xxxi.

⁶³ See this vol., p. 226.

⁶⁴ R. J. Williams, H. K. Mitchell, H. H. Weinstock, and E. E. Snell, *J. Amer. Chem. Soc.*, 1940, **62**, 1784.

⁶⁵ E. T. Stiller, S. A. Harris, J. Finkelstein, J. C. Keresztesy, and K. Folkers, *ibid.*, p. 1785.

⁶⁶ D. W. Woolley, *ibid.*, p. 2251.

⁶⁷ H. H. Weinstock, A. Arnold, E. L. May, and D. Price, *Science*, 1940, **91**, 411; S. H. Babcock and T. H. Jukes, *J. Amer. Chem. Soc.*, 1940, **62**, 1628.

activity, and (-)pantothenic acid is inactive;⁶⁵ the hydroxy-derivative⁶⁸ has a variable potency depending on conditions, but various other synthetic analogues are biologically inert.⁶⁹

The pathological and physiological relations of pantothenic acid are now receiving attention. In chicks,⁷⁰ a deficiency results in degeneration of the nerve fibres of the spinal cord. In rats,⁷¹ as well as the better known symptoms⁵⁸ such as nose bleeding, sticky exudate on eyelids, and depilation about the nose, the adrenals are said to suffer haemorrhage, atrophy, and necrosis. The tissues of deficient chicks have been shown to be low in the vitamin;⁷² and a method has been suggested for estimating it in human blood.⁷³

The conclusion given in last year's Report about the identity of the "filtrate factor," needed by rats, with pantothenic acid has been further confirmed.⁷⁴ Associated with the filtrate factor are other substances also needed by rats, *viz.*, the so-called β - and γ -factors of the British workers⁷⁴ and the Factor W of Elvehjem and his collaborators.⁷⁵

Vitamin C.

Deficiency in Man.—Working details have been given of the simplified procedure for assessing the level of nutrition of vitamin C in human subjects, by means of the urine test: controls at a home for waifs and strays in Cambridge where an orange was provided daily were found to be up to standard, whereas in poor-class homes in the same town some 40% of the children were "below standard" and 5% had a relatively severe deficiency.⁷⁶ When it is merely desired to distinguish in a rough qualitative way between children with high and with low "reserves," it is possible to use a still simpler modification of the test.⁷⁷ In polar regions, the large consumption of fresh meat, and at some seasons of arctic flora, generally keeps the

⁶⁸ H. K. Mitchell, E. E. Snell, and R. J. Williams, *J. Amer. Chem. Soc.*, 1940, **62**, 1791.

⁶⁹ H. H. Weinstock, E. L. May, A. Arnold, and D. Price, *J. Biol. Chem.*, 1940, **135**, 343.

⁷⁰ P. H. Phillips and R. W. Engel, *J. Nutrition*, 1939, **18**, 227; quoted by *Brit. Med. J.*, 1940, **2**, 230.

⁷¹ F. S. Daft, W. H. Sebrell, S. H. Babcock, and T. H. Jukes, *Publ. Health Reps., Wash.*, 1940, **55**, 1333; L. L. Ashburn, *ibid.*, p. 1337.

⁷² E. E. Snell, D. Pennington, and R. J. Williams, *J. Biol. Chem.*, 1940, **133**, 559.

⁷³ S. R. Stanbery, E. E. Snell, and T. D. Spies, *ibid.*, 1940, **135**, 353.

⁷⁴ B. Lythgoe, T. F. Macrae, R. H. Stanley, A. R. Todd, and C. E. Work, *Biochem. J.*, 1940, **34**, 1335.

⁷⁵ S. Black, D. V. Frost, and C. A. Elvehjem, *J. Biol. Chem.*, 1940, **132**, 65; J. J. Oleson and S. Black, *ibid.*, 1940, **133**, proc. lxxiii.

⁷⁶ L. J. Harris, *Lancet*, 1940, ii, 259.

⁷⁷ J. Pemberton, *Brit. Med. J.*, 1940, **2**, 217.

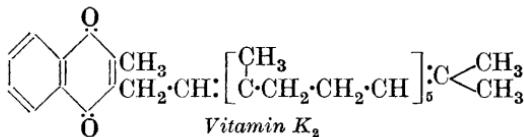
people free from scurvy,⁷⁸ but certain Eskimos on a diet devoid of meat were found to be deficient in the vitamin when tested either for the ascorbic acid in their blood or for capillary resistance—both could be improved if orange juice were given.⁷⁹ Other signs of deficiency included rhinitis, oedematous nasal mucous membranes, epistaxis and increased severity of the cutaneous response to tuberculin.^{79, 80} The increased importance of an adequate supply of vitamin C in war-time is illustrated by the fact that the healing of wounds and the formation of callus are delayed by partial deficiencies.⁸¹ Those working on the apparent connection between vitamin C and resistance to infection may receive further clues from the observation concerning the tuberculin reaction mentioned above, and from the finding of the workers at Pittsburg that the administration of hypnotics such as barbiturates or chloretone caused an increased synthesis of the vitamin by rats, attributable, it is presumed, to its connection with detoxication processes.⁸²

Properties and Distribution of Ascorbic Acid.—A valuable compilation has been made by M. Olliver⁸³ of the distribution of vitamin C in numerous raw and cooked fruits and vegetables. C. L. Arcus and S. S. Zilva⁸⁴ have examined the photochemical decomposition of ascorbic acid and conclude that "it is improbable that any ultra-violet light penetrating superficial tissues containing *l*-ascorbic acid would bring about the oxidation of the vitamin *in vivo*."

Other Vitamins.

Owing to lack of space this year, a discussion of recent work on vitamins D and E will have to be postponed until the next volume.

Vitamin K.—The formula given provisionally for vitamin K₂ in



last year's Report has been modified. Instead of two farnesyl residues, one each in the 2- and the 3-position, it is now thought that

⁷⁸ K. Rodahl, private communication.

⁷⁹ V. E. Levine, *J. Biol. Chem.*, 1940, **133**, proc. lxi.

⁸⁰ A. Høyaard, *Nord. med. Tidsskr.*, 1938, **16**, 1647. For further reference to the tuberculin reaction in vitamin C deficiency, see also K. M. Birkhaug, *Acta tuberc. Scand.*, 1939, **13**, 45; quoted by V. E. Levine (*loc. cit.*).

⁸¹ E. W. Lexer, *Arch. klin. Chir.*, 1939, **195**, 611.

⁸² H. E. Longenecker, H. H. Fricke, and C. G. King, *J. Biol. Chem.*, 1940, **135**, 497.

⁸³ *Lancet*, 1940, ii, 190.

⁸⁴ *Biochem. J.*, 1940, **34**, 61.

the two are combined, joined head to tail, in the 3-position.⁸⁵ According to this view vitamin K₂ is 3-difarnesyl-2-methyl-1 : 4-naphthaquinone. This brings K₂ into line with all the more potent K-factors, in having a methyl substituent in the 2-position.

“Vitamin P.”—The claims for “vitamin P” have again been controverted, so far as concerns the guinea pig;⁸⁶ for the human some clinical observers record positive⁸⁷ and others negative results.⁸⁸ We may reasonably hope for an explanation of the discrepancy within a year or two.

Vitamin F (Essential Unsaturated Fatty Acids).—The previous indications given by T. W. Birch and his co-workers of inter-relations between vitamin B₆ and the nutritionally essential fatty acids have been confirmed: symptoms of so-called acrodynia in the rat may, it seems, be cured by either factor independently.⁸⁹ Possibly, however, the symptoms in the two deficiencies are similar but not identical; indeed G. O. Burr, J. B. Brown, and W. O. Lundberg⁹⁰ say that the unsaturated fatty acids themselves (linolenic, linoleic, arachidonic, etc.) should not be treated as an interchangeable group, since they differ appreciably in their effects on growth and on the skin lesions. Interesting studies of the relative potencies of various esters of these fatty acids, and of their effects on fat metabolism, have been made by (Mrs.) I. Smedley-Maclean and her colleagues at the Lister Institute.⁹¹

Vitamin H.—Vitamin H is the substance which protects rats against “egg-white injury,” that is to say, against the symptoms produced by consumption of large amounts of raw or of insufficiently cooked egg-white.^{91a} Investigations on this vitamin were begun by P. György in Heidelberg in 1927, were resumed with T. W. Birch in Cambridge from 1933—1935, and have since been continued in conjunction with others in America. A full resumé of all these extended experiments has appeared.⁹² One of the most interesting

⁸⁵ S. B. Binkley, R. W. McKee, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, 1940, **133**, 721.

⁸⁶ L. E. Detrick, M. S. Dunn, W. L. McNamara, and N. E. Hubbard, *J. Lab. Clin. Med.*, 1940, **25**, 684.

⁸⁷ H. Scarborough, *Biochem. J.*, 1939, **33**, 1400; *Lancet*, 1940, ii, 644; D. R. Gorrie, *ibid.*, i, 1005.

⁸⁸ *E.g.*, E. Davis, *ibid.*, p. 1062.

⁸⁹ H. Schneider, H. Steenbock, and B. R. Platz, *J. Biol. Chem.*, 1940, **132**, 539.

⁹⁰ *Proc. Soc. Exp. Biol. Med.*, 1940, **44**, 242.

⁹¹ E. M. Hume, L. C. A. Nunn, I. Smedley-MacLean, and H. H. Smith, *Biochem. J.*, 1940, **34**, 879; I. Smedley-MacLean and L. C. A. Nunn, *ibid.*, p. 884; G. C. Hevesy and I. Smedley-MacLean, *ibid.*, p. 903.

^{91a} M. A. Boas, *ibid.*, 1927, **21**, 712.

⁹² P. György, *J. Biol. Chem.*, 1939, **131**, 733; P. György, R. Kuhn, and E. Lederer, *ibid.*, p. 745; T. W. Birch and P. György, *ibid.*, p. 761.

points is that vitamin H as it occurs in yeast or liver is insoluble in water or fats, but can be made soluble in water by autolysis (in the case of yeast) or by a suitable process of digestion (in the case of liver). It is three to five times more effective when given parenterally than by mouth.⁹³ Lastly the important suggestion has been made that vitamin H is probably identical^{94, 95} with biotin ("bios II B")—a factor needed for the growth of yeast and by certain strains of *S. aureus*—and also with "Coenzyme R"⁹⁴—a growth factor needed by nodular organisms in certain plant roots.

New Vitamins.—The casual reader of current literature on vitamins will probably be mystified by allusions to such novel terms as the grass-juice factor, the anti-grey-hair factor, vitamins L and M, etc. Most of these newly described substances are as yet but poorly characterised chemically, but for enumeration and classification reference may be had to a recent review.⁹⁶

Nutrition in War-time.

In Britain the nutritional problem is, in essence, how to secure adequate nutrition while reducing the importation of food to the minimum. The solution lies, mainly, in an increased reliance on commodities already produced in large quantities at home. Potatoes and milk come foremost in this category. Together these two foods constitute a regimen which is not far from complete as regards most of the vitamins, mineral salts, and protein; and enough of each is available to furnish a considerable proportion of the daily food requirements of the population.⁹⁷ Examples of two other home-produced foodstuffs which are being encouraged are carrots, valuable as a source of vitamin A, and oatmeal, also a native crop and good for cheap calories and for vitamin B₁. The "nutrition front" must be of supreme interest to every chemist and biochemist just now, as an instance where applied chemical and biochemical knowledge has become of fundamental importance to the national life. The theme, unfortunately, cannot be more than vaguely outlined here, and we must content ourselves with a cross reference to some more detailed recent writings.⁹⁸

⁹³ P. György and C. S. Rose, *Proc. Soc. Exp. Biol. Med.*, 1940, **43**, 73.

⁹⁴ P. György, D. B. Melville, D. Burk, and V. du Vigneaud, *Science*, 1940, **91**, 243.

⁹⁵ J. R. Porter and M. J. Pelczar, *ibid.*, p. 576.

⁹⁶ L. J. Harris, *Post Grad. Med. J.*, 1941, **17**, 34.

⁹⁷ For discussion on the potato as a war-time food, see R. N. Salaman and others in contributions to a symposium reported in *Chem. and Ind.*, 1940, **59**, 735 *et seq.*

⁹⁸ E.g., Sir J. B. Orr and D. Lubbock, "Feeding the People in War-Time," 1940; L. J. Harris, *Practitioner*, 1940, **145**, 105; J. C. Drummond, Sir R.

White versus Brown Bread.—Nutrition workers are unanimous in recognising wholemeal flour, or flour of a similar high degree of "extraction," as superior to white flour. The proposal to fortify white bread by adding to it crystalline aneurin plus a calcium salt, stated to be the carbonate, has been discussed by many authors.^{99, 100} The addition of vitamin B₁ and calcium does not, however, make good all the deficiencies of white flour as compared with wheatmeal, and this has been convincingly confirmed in growth tests on rats by H. Chick.¹⁰¹

L. J. H.

3. IMMUNOCHEMISTRY.

Bacterial Antigens.

G. G. Freeman, S. W. Challinor, and J. Wilson¹ have compared the antigens isolated from members of the *Salmonella* group (*Bact. Typhi-murium* and *Bact. Typhosus*) by the methods of A. Boivin and L. Mesrobeanu,² and R. Raistrick and W. W. C. Topley³ and by extraction with ethylene glycol and diethylene glycol.⁴ They also prepared the antigens from bacteria grown on a synthetic medium. J. Walker⁵ used a method of extraction with concentrated solutions of urea. The four methods gave very similar products. G. M. Mackenzie, R. H. Pike, and R. E. Swinney⁶ also found that similar products were obtained by the methods of Raistrick and Topley and of Boivin and Mesrobeanu. It may be supposed that the antigenic material obtained by these diverse methods exists as a complex in the bacteria and is not a chance mixture of various contents of the bacterial cell and the culture made.

Morgan⁴ extracted from *Bact. Dysenteriae* (Shiga) an antigen

McCarrison, Sir J. B. Orr, Sir F. Keeble, L. H. Lampitt, V. H. Mottram, J. C. Spence, and F. Kidd, "The Nation's Larder . . .," 1940; G. Bourne, "Nutrition and the War," 1940; Sir J. Russell, *Nature*, 1940, **145**, 11. See also the valuable tabulations by A. L. Bacharach and J. C. Drummond, *Chem. and Ind.*, 1940, **59**, 37, on the human requirements for vitamins, expressed both as marginal and as optimal limits, and by A. L. Bacharach, *Food*, 1940, **9**, 110, on the contributions made by the more important foodstuffs towards these requirements.

⁹⁹ *E.g.*, Accessory Food Factors Committee, *Lancet*, 1940, ii, 143; W. C. McCulloch, *Brit. Med. J.*, 1940, **2**, 397; J. P. McGowan, *ibid.*, p. 398; (Sir) E. Graham-Little, *Lancet*, 1940, ii, 311; A. L. Bacharach, *Food Manufacture*, 1940, **15**, 220; E. R. Dawson, *Chem. and Ind.*, 1940, **59**, 784.

¹⁰⁰ T. Moran and J. C. Drummond, *Nature*, 1940, **146**, 117.

¹⁰¹ *Lancet*, 1940, ii, 511. ¹ *Biochem. J.*, 1940, **34**, 307.

² *Compt. rend. Soc. Biol.*, 1933, **112**, 76.

³ *Brit. J. Exp. Path.*, 1934, **15**, 114.

⁴ W. T. J. Morgan, *Biochem. J.*, 1937, **31**, 2503.

⁵ *Ibid.*, 1940, **34**, 325.

⁶ *J. Bact.*, 1940, **40**, 197.

(ABC) composed of a phospholipin (A), a polysaccharide (B), and a polypeptide (C). W. T. J. Morgan and S. J. Partridge⁷ consider that the product is a homogeneous complex, which can induce the formation of antibodies like the intact organism. If A is removed, BC is still antigenic (induces formation of antibodies on injection), but A alone and AB are not. C on injection into rabbits gives rise to antibodies that react with C or BC; however, these do not appear to be the same as those evoked by BC or the intact organism. BC can be regenerated from isolated B and C. The purified polysaccharide B forms precipitates with antisera to the bacteria.

To obtain a bacterial antigen with the minimum disintegration A. A. Miles and N. W. Pirie⁸ extracted *Br. Melitensis* with chloroform water. They obtained a substance [PLAPS], from which a phospholipin PL, and a protein-like substance S could be successively removed, leaving AP. Hydrolysis with weak hydrochloric acid split AP, liberating a phospholipin and an *N*-formylated amino-polyhydroxide compound A, which may be a formylated amino-hexose. [PLAPS], which forms viscous opalescent solutions that show anisotropy of flow, is split on treatment with sodium dodecyl sulphate into smaller particles, PLAPS, with molecular weights between 10^5 and 10^6 . AP has a molecular weight of about 10^6 , which can be reduced to between 1 and 2×10^5 . The amine, freed from formic acid, is fairly homogeneous,⁹ with a minimum particle weight of 3300.

The ability to form antibodies on injection (antigenicity) is in the order [PLAPS] > PLAPS > AP; the antigenicity of AP may be due to contamination with traces of PLAPS. [PLAPS] forms more precipitate with antisera than PLAPS or AP. A is not antigenic and forms no precipitate with antisera. These substances, when added in excess, inhibit the agglutination of *Br. Melitensis* by homologous antisera. The concentrations required for inhibition are in the order Free amine \gg A > [PLAPS] > AP.

These observations illustrate with single natural antigens the dependence of antigenicity and the formation of precipitates with antisera on the degree of aggregation and complexity of the complex, and of the specificity on a relatively simple fraction of the antigen.

Natural Protein Antigens.

Catalase.—Antisera to crystalline ox catalase have been obtained by immunising rabbits.¹⁰ Dog and horse catalase also formed pre-

⁷ *Biochem. J.*, 1940, **34**, 169.

⁸ *Brit. J. Exp. Path.*, 1939, **20**, 83, 109, 278; *Biochem. J.*, 1939, **33**, 1709, 1716.

⁹ J. St. L. Philpot, *Biochem. J.*, 1939, **33**, 728.

¹⁰ D. A. Campbell and L. Fourt, *J. Biol. Chem.*, 1939, **129**, 383.

cipitates with the antiserum; but haemoglobin and haematin neither formed precipitates with the antiserum nor inhibited the formation of a precipitate by catalase and antiserum. The specificity of the catalase is, therefore, not determined by the iron-porphyrin group.

When the catalase was precipitated in combination with antibody, its enzyme activity was unimpaired; the specific groups that combine with antibody are not the same as those involved in the enzyme activity.

Bence Jones Proteins.—L. Hektoen and W. H. Welker¹¹ have confirmed conclusions from previous work that Bence Jones proteins fall into two groups, which are immunologically distinct. Both types may be excreted by the same patient. They report that Medes has found the nitrogen distribution similar in all but one of their preparations. The immunological behaviour of this one resembled that of other preparations from which it differed chemically. This suggests that the specificity of these proteins is determined by a small part of the protein molecule.

L. Pillemer and others¹² have studied the specificity of keratin derivatives. They find a difference between feather and wool keratin and consider that the S-C-CO₂H group is of prime importance in determining specificity.

Synthetic Antigens.

W. F. Goebel¹³ has continued his investigations¹⁴ on the rôle of uronic acids in the immunity to pneumococci. He found that synthetic antigens containing gentioburonic and celloburonic acids linked to horse serum globulin (GeA- and CA-globulin) evoked in rabbits antibodies which conferred passive immunity on mice against multiple doses of Type II pneumococci. However, antisera to GeA-globulin did not protect mice against Type III and Type VIII pneumococci, as did antisera to CA-globulin.¹⁴ The specific polysaccharides of Type III and Type VIII pneumococci contain celloburonic acids; the uronic acid constituent of Type II polysaccharide is still unknown. The cross reactions of GeA- and CA-globulins and of similar compounds containing gentiobiose were studied. The point of attachment of the glucose or glucuronic acid to the glucose seemed of more importance than the presence of a carboxyl group.

Further investigations have been made into the effects of substitution in the amino-group of proteins on their immunological behaviour. If the amino-group of a protein P₁ (*e.g.*, horse serum globulin) is treated to form P₁R, will (1) P₁R still form a precipitate

¹¹ *Biochem. J.*, 1940, **34**, 487.

¹² *J. Exp. Med.*, 1939, **70**, 287.

¹³ *Ibid.*, 1940, **72**, 33.

¹⁴ *Ibid.*, 1939, **69**, 33.

with antisera to the untreated protein P_1 , (2) P_1 form a precipitate with antisera to P_1R , (3) the product P_2R , formed with another protein P_2 (*e.g.*, egg albumin), form a precipitate with antisera to P_1R , and (4) the formation of a precipitate by P_2R and antisera to P_1R be inhibited by the relatively simple compounds (AR) formed by treating amino-groups of amino-acids?

Formaldehyde Treatment (P_1F).—L. J. Jacobs and S. C. Gommers¹⁵ compared the effects of the various methods of treatment with formaldehyde that have been used by previous workers. They found that P_1F formed precipitates with antisera to P_1 , and P_1 with antisera to P_1F ; P_2F formed little or no precipitate with antisera to P_1F .

A. B. Kleczkowski,¹⁶ using quantitative methods in place of the traditional inspection and rows of + signs, found that P_1F precipitated all the antibody from antisera to P_1 .

Phenylureidoproteins (PU).¹⁷ Kleczkowski found that P_1U also precipitated all the antibody from antisera to P_1 . P_2U precipitated only part of the antibody from antisera to P_1U , which did not react with P_1 .

Diethyl sulphide and diethylsulphone proteins (PMS and PMSO). L. Berenblum and A. Wormall¹⁸ treated horse serum globulin with $\beta\beta'$ -dichlorodiethyl sulphide and $\beta\beta'$ -dichlorodiethylsulphone, forming P_1MS and P_1MSO . P_1MS formed a slight precipitate with antisera to P_1 , and P_1 with antisera to P_1MS . P_2MS and P_2MSO reacted weakly with antisera to P_1MS and P_1MSO respectively.

Carbobenzyloxyproteins (PC). P_2C formed precipitates with antisera to P_1C . This reaction was inhibited by carbobenzyloxy-glycine and the rather similar phenylureidoglycine.¹⁹

Aspirylproteins (PA). G. C. Butler, C. R. Harrington, and M. E. Yuill (working with Miles)²⁰ found that P_2A reacted with antisera to P_1A ; the reaction was inhibited by aspirylglycine and to a less degree by salicylglycine.

These experiments show that the specificity of proteins is little affected when the amino-group is changed by treatment with formaldehyde; but that when larger groups are attached at this site, the specificity is altered. They suggest that the amino-groups have little relation to the immunological behaviour of proteins and that when a new specificity is introduced it is not due to loss of the free amino-group but to the presence of large new groups.

¹⁵ *J. Immun.*, 1939, **36**, 531. ¹⁶ *Brit. J. Exp. Path.*, 1940, **21**, 1.

¹⁷ S. J. Hopkins and A. Wormall, *Biochem. J.*, 1933, **27**, 740; 1934, **28**, 228.

¹⁸ *Ibid.*, 1939, **33**, 75.

¹⁹ W. E. Gaunt and A. Wormall, *ibid.*, p. 908.

²⁰ *Ibid.*, 1940, **34**, 838.

According to A. B. Kleczkowski,²¹ in the course of iodination of horse serum globulin the ability to react with and the ability to evoke antibodies to native globulin disappear when all the tyrosin is substituted.

Butler, Harrington, and Yuill²⁰ also found that injection of antisera to P₁A reduced the antipyretic action of aspirin (compare a similar effect with thyroxin; *Chem. Reviews*, 1938, 356).

Antibodies.

The recognition that antibodies are modified serum globulins and the new methods of characterising proteins have led to considerable work on the physical properties of antibodies. A. Tiselius²² found that normal serum globulin consisted of three fractions, whose mobilities in an electric field at p_{H} between 7 and 8 were in the order $\alpha > \beta > \gamma$. Horse antisera may contain either an excess of the γ -fraction or a new fraction, T, with a mobility between those of the β - and the γ -fraction. When the antibody is removed by absorption with the corresponding antigen, the γ -fraction is reduced or the γ -fraction removed. In the sera of men, monkeys, and rabbits, antibodies were found mainly in the γ -fraction.^{22, 23, 24}

According to J. van der Scheer, R. W. G. Wyckoff, and F. H. Clarke²⁵ all horse antisera to toxins contain the new fraction T, whereas horse antisera to bacterial polysaccharides and proteins contain abnormal amounts of the γ -fraction. Some antitoxic sera contain both T-fraction and excess of γ -fraction. These authors suggest that these two fractions may contain antibodies to two different antigens present in the complex substance used for immunisation. Pappenheimer, Lundgren, and Williams²⁶ also found that diphtheria antitoxin formed a new serum globulin fraction. However, Tiselius and Kabat²³ found that the antibody of horse antisera to pneumococcal polysaccharide was in the T-fraction, and N. Fell, W. G. Stern, and R. D. Coghill²⁶ found no abnormal fraction in antisera to various bacterial toxins.

Using the ultra-centrifuge, Biscoe, Hercik, and Wyckoff²⁴ and H. Heidelberger and K. O. Pedersen²⁷ showed that purified antibodies, from horse antisera to the pneumococcal polysaccharides, had very high sedimentation constants, whereas antibodies of rabbit antisera both to pneumococcal polysaccharides and to proteins had sedimentation constants close to those of normal serum globulin. E. A. Kabat²⁸ extended this work; he contrasted the antibodies

²¹ *Brit. J. Exp. Path.*, 1940, **21**, 98. ²² *Biochem. J.*, 1937, **31**, 1464.

²³ A. Tiselius and E. A. Kabat, *J. Exp. Med.*, 1939, **69**, 119.

²⁴ J. Biscoe, F. Hercik, and R. W. G. Wyckoff, *Science*, 1936, **83**, 602.

²⁵ *J. Immun.*, 1940, **39**, 65 (references are given to previous work).

²⁶ *Ibid.*, p. 223. ²⁷ *J. Exp. Med.*, 1937, **65**, 393. ²⁸ *Ibid.*, 1939, **69**, 103.

found in the sera of horses, cows, and pigs, which are very large (molecular weight about 990,000) and unsymmetrical, with those of the sera of men, monkeys, and rabbits which resemble normal serum globulin (molecular weight 157,000 to 195,000). A. M. Pappenheimer (jun.), H. P. Lundgren, and J. W. Williams,²⁹ however, found that the molecular weight of diphtheria antitoxin in horse serum is about 155,000; and Fell, Stern, and Coghill,²⁶ that horse antisera to various bacterial toxins contain no proteins of large molecular weight. Kabat²⁸ also found that, on prolonged immunisation of horses against pneumococci, smaller antibody molecules were formed. So that it seems that there is no inherent difference between antibodies either in molecular weight or in charge, dependent on the species in which they are formed or on the antigen with which they react.

Enzymic Digestion.—Quantitative experiments were made by A. M. Pappenheimer (jun.) and E. S. Robinson³⁰ with the diphtheria antitoxin prepared by the Parfentjev digestion process. These suggest that the antibody molecules are split by the enzyme into an active and an inactive part. Similar work by P. Grabar³¹ indicates that the antibody to pneumococcal polysaccharides is split by pepsin into two approximately equal parts, one active and the other inactive. C. G. Pope³² studied the effect of varying conditions, p_H , time of digestion, etc., on the purification of antibody by digestion with proteolytic enzymes. He finds that antibodies are readily split by digestion in a very short time into an inactive portion, which is easily denatured by heat, and an active portion more resistant to heat. Ordinary antitoxin contains 60,000—90,000 units, and enzyme-treated antitoxin about 140,000 units, per gram of protein (also found by Pappenheimer and Robinson). Continuation of this work would shed light on the structure of proteins and the peculiarities of the structure of antibodies.

The practical value of this method lies in the elimination of non-specific proteins, which make up some 90% of the proteins of an untreated serum. The results can be judged by the number of units of antitoxin per gram of protein in the product, which may be increased eight times. Various techniques depending on long digestion have proved less satisfactory.^{33, 34, 35} Since the digested

²⁹ *J. Exp. Med.*, 1940, **71**, 247.

³⁰ *J. Immun.*, 1937, **32**, 291.

³¹ *Ann. Inst. Pasteur*, 1938, **61**, 765.

³² *Brit. J. Exp. Path.*, 1939, **20**, 132, 201, 213.

³³ F. Modern and G. Ruff, *Compt. rend. Soc. Biol.*, 1938, **129**, 851; *Biochem. Z.*, 1938, **299**, 377.

³⁴ F. Hansen, *Compt. rend. Soc. Biol.*, 1938, **129**, 216; *Biochem. Z.*, 1938, **299**, 363.

³⁵ G. Sandor, *Compt. rend. Soc. Biol.*, 1939, **130**, 840, 1187.

antibody appears not to be antigenic,³⁷ injection of it should not cause serum sickness or anaphylaxis, which, however, is a very rare accident in man. The method can also be applied to tetanus antitoxin.³⁶

The Reaction between Antibodies and Antigens.

Knowing the molecular weights of antibody (A) and antigen (G), it is possible to calculate the composition of the aggregate formed by their interaction; this has been done by M. Heidelberger³⁸ and A. M. Pappenheimer (jun.), H. P. Lundgren, and J. W. Williams.³⁹ Theories of the reaction between A and G suppose that large soluble aggregates are formed when G is in great excess. The formation of such aggregates has been shown by the ultra-centrifuge.

The reactions of A and G when spread in films on surfaces have been studied.⁴⁰ Measurements of the thickness of films on solid surfaces have given objective demonstration of the specific union of A and G, proof that a molecule of A can combine with more than one molecule of G, and vice versa, and dimensions of A and G molecules that agree fairly well with those obtained by other methods. Some discrepancies may be due to unequal spreading and to the use of different methods of spreading and different types of surface.

When antibodies to the bacterial polysaccharides are spread on water to form a film, one amino-acid thick, they do not combine with the corresponding polysaccharides. In films on solid surfaces, the antibodies preserve a "globular" form and combine with polysaccharide.

J. R. M.

4. HORMONES.

Since the anterior pituitary hormones have not been discussed in these Reports lately, this section will deal exclusively with the more important investigations on this gland, the consideration of other endocrine organs being kept over for later reports. Even so, it is obviously impossible, in one brief section, to mention all contributions concerning this particular gland.

³⁶ G. Sandor and R. Richou, *Compt. rend. Soc. Biol.*, 1939, **131**, 461.

³⁷ A. J. Weil, I. A. Parfentjev, and K. L. Bowman, *J. Immun.*, 1938, **35**, 399.

³⁸ *J. Amer. Chem. Soc.*, 1938, **69**, 103.

³⁹ *J. Exp. Med.*, 1940, **71**, 247.

⁴⁰ I. Langmuir and V. J. Schaefer, *J. Amer. Chem. Soc.*, 1937, **59**, 1406; J. F. Danielli, J. M. Danielli, and J. R. Marrack, *Brit. J. Exp. Path.*, 1938, **19**, 393; M. E. Shaffer and J. H. Dingle, *Proc. Soc. Exp. Biol. Med.*, 1938, **38**, 528; A. Rothan and K. Landsteiner, *Science*, 1939, **40**, 65; E. F. Porter and A. M. Pappenheimer (jun.), *J. Exp. Med.*, 1939, **69**, 755.

Anterior Pituitary.

Gonadotrophic Hormones.—Several workers have recently succeeded in separating distinct follicle-stimulating (FSH) and luteinising fractions (LH) from pituitary extracts, using the relative insolubility of the luteinising hormone in ammonium sulphate solution at p_{H} 4.2 or 5—6.¹ These results were confirmed by biological assays.² The LH is apparently identical with the hormone of Evans, stimulating the interstitial cells of the testes and ovaries (ICSH).³

The follicle-stimulating hormone (FSH) contained in unfractionated pituitary extracts is a protein complex whose activity is, according to several laboratories (with the exception of A. A. Abramowitz and F. L. Hisaw⁴), relatively unaffected by digestion with commercial or crystalline trypsin, while the luteinising potency (LH) is destroyed.⁵ FSH was found to be very rich in carbohydrate (principally glucosamine) when compared with the ICSH (or LH).⁶ From acetylation by keten of these sugar-rich proteins, C. H. Li, M. E. Simpson, and H. M. Evans conclude that the activity of the FSH and LH is dependent on the free amino-groups.⁷ H. Fraenkel-Conrat *et al.*, contrary to recent results of F. Bischoff,⁸ found that cysteine destroys all gonadotrophic activity of pituitary hormones, acting upon the supposed S-S linkings considered to be essential for their potency. The chorionic gonadotrophic hormone (CGH, prolan from human pregnancy urine), however, showed no loss of activity.⁹ For these and other reasons, CGH is regarded as different from pituitary luteinising hormone, with which it has some biological effects in common. CGH (of placental origin) resembles insulin in that the phenolic hydroxyls rather than the free amino-groups are essential for its activity.⁷ CGH is apparently a gluco-

¹ H. L. Fevold, *Endocrinology*, 1939, **24**, 435; H. Jensen, M. E. Simpson, S. Tolksdorf, and H. M. Evans, *ibid.*, 1939, **25**, 57; H. Jensen, S. Tolksdorf, and F. Bamman, *J. Biol. Chem.*, 1940, **135**, 791; R. O. Greep, H. B. Van Dyke, and B. F. Chow, *ibid.*, 1940, **133**, 289; H. Rinderknecht and P. C. Williams, *J. Endocrinol.*, 1939, **1**, 117.

² H. M. Evans, M. E. Simpson, S. Tolksdorf, and H. Jensen, *Endocrinology*, 1939, **25**, 529.

³ H. L. Fevold, *J. Biol. Chem.*, 1939, **128**, 83.

⁴ *Endocrinology*, 1939, **25**, 529.

⁵ W. H. McShan and R. K. Meyer, *J. Biol. Chem.*, 1938, **126**, 361; G. Chen and H. B. Van Dyke, *Proc. Soc. Exp. Biol. Med.*, 1939, **42**, 454; R. C. Li, *ibid.*, 1940, **43**, 598; B. F. Chow, R. O. Greep, and H. B. Van Dyke, *J. Endocrinol.*, 1939, **1**, 440.

⁶ H. M. Evans, H. Fraenkel-Conrat, M. E. Simpson, and C. H. Li, *Science*, 1939, **89**, 249; W. H. McShan and R. K. Meyer, *J. Biol. Chem.*, 1940, **135**, 473.

⁷ *J. Biol. Chem.*, 1939, **131**, 259. ⁸ *Ibid.*, 1940, **134**, 641.

⁹ H. Fraenkel-Conrat, M. E. Simpson, and H. M. Evans, *ibid.*, 1939, **130**, 243; *Science*, 1940, **91**, 363.

protein (4 RU per 1 $\mu\text{g}.$), the carbohydrate of which consists of hexosamine-digalactose units. The amino-group of the hexosamine is probably acetylated, and another acetyl group is attached to another part of the molecule. The minimal molecular weight lies between 60,000 and 80,000. The isoelectric point was found to be at p_{H} 3.2—3.3.¹⁰

The isolation of the interstitial cell stimulating (or luteinising) hormone in pure form (1 unit in 5 $\mu\text{g}.$) has recently been claimed by two laboratories.^{11, 12} Although the isolated proteins have similar biological properties, the results from the electrophoretic study are quite different. The isoelectric point is stated to be at p_{H} 7.45 and 4.6, respectively, and the mobility of the protein 6.36×10^{-5} and 0.66×10^{-5} , respectively. The gonadotrophic protein of C. H. Li *et al.* contained 4.45% of mannose, 5.86% of glucosamine, and 14.2% of nitrogen with approximately 4.5% of tyrosine and 1% of tryptophan.

Thyrotrophic Hormone.—Various methods for the evaluation of the thyrotrophic potency of pituitary extracts have been proposed, of which Q. K. Smelser's test based on the increase of the thyroid weight of the one-day-old chicks may be mentioned.¹³ Recent reports of the isolation of nearly pure thyrotrophic hormone with a negligible luteinising potency¹⁴ do not support the claim that the thyrotrophic is a property of the luteinising hormone rather than due to a separate entity.¹⁵ J. Fraenkel-Conrat *et al.* found that this active protein contains 13% of nitrogen, 3.5% of carbohydrate, and 2.5% of glucosamine. Cysteine and keten treatment inactivates the hormone. Some metabolic effects of pituitary extracts, however, cannot be ascribed to the thyrotrophic hormone and seem to be independent of the thyroid.¹⁶

¹⁰ S. Gurin, C. Bachman, and D. W. Wilson, *J. Biol. Chem.*, 1939, **128**, proc. xxxvii, 525; 1940, **133**, 467, 477; *J. Amer. Chem. Soc.*, 1939, **61**, 2251.

¹¹ T. Shedlovsky, A. Rothen, R. O. Greep, H. B. Van Dyke, and B. F. Chow, *Science*, 1940, **92**, 178.

¹² C. H. Li, M. E. Simpson, and H. M. Evans, *ibid.*, p. 355.

¹³ Q. K. Smelser, *Proc. Soc. Exp. Biol. Med.*, 1937, **38**, 388; *Endocrinology*, 1938, **23**, 429.

¹⁴ C. G. Lamble and V. M. Trikojus, *Biochem. J.*, 1937, **31**, 843; B. F. Chow, R. O. Greep, and H. B. Van Dyke, *J. Endocrinol.*, 1939, **1**, 440; H. L. Fevold, M. Lee, F. L. Hisaw, and E. J. Cohn, *Endocrinology*, 1940, **26**, 999; R. W. Bonsnes and A. White, *ibid.*, p. 990; J. Fraenkel-Conrat, H. Fraenkel-Conrat, M. E. Simpson, and H. M. Evans, *J. Biol. Chem.*, 1940, **135**, 235, 199.

¹⁵ H. Jensen and J. F. Grattan, *Amer. J. Physiol.*, 1937, **37**, 388; H. Jensen and S. Tolksdorf, *Endocrinology*, 1939, **25**, 429; *Proc. Soc. Exp. Biol. Med.*, 1939, **42**, 466.

¹⁶ D. K. O'Donovan and J. B. Collip, *Canadian Med. Assoc. J.*, 1938, **39**, 83; H. H. Neufeld and J. B. Collip, *ibid.*, p. 83; O. F. Denstedt and J. B. Collip, *ibid.*, p. 84.

Growth Hormone.—The growth of "plateaued" or hypophysectomised animals has been used for assays of the growth potency of pituitary extracts.¹⁷ Recently J. Freud and L. H. Levie found that the growth of the tail and development of caudal vertebrae of hypophysectomised young rats ceased altogether. They use this effect upon the proliferating zone of the cartilage for assays by making X-ray pictures and histological examination of the tails.¹⁸ However, thymus extracts also give a positive result in this test.¹⁹ The evidence that there is a pituitary hormone specifically concerned with growth is convincing. Preparations of the growth hormone can be almost freed from thyrotrophic (another growth-promoting factor) and lactogenic hormone by treatment with cysteine.²⁰ Highly purified preparations were obtained (active in doses of 3—10 µg.) having the elementary composition: C, 49.76; H, 7.24; N, 14.27; S, 1.47% (in dithio-groups). They were labile in heat, acid, and alkali, and were destroyed by trypsin and pepsin. An absorption maximum was observed at 2830 Å.²¹

Lactogenic Hormone.—An extensive review of this hormone has appeared.²² All evidence indicates that the factor which initiates lactation in mammals is identical with the pigeon crop stimulating factor. Upon this fact are based most methods of assay.²³ Several workers have attempted a separation of prolactin.²⁴ The crystalline product of protein nature, the lowest effective dose of which was found to be 0.1—0.2 µg. per pigeon, contained C, 51.11; H, 6.76; N, 14.38; S, 1.77%; tryptophan, tyrosine, and phenylalanine were identified in the preparation.²⁵ The isoelectric point was at p_H 5.7 and its mobility $d\mu/dp_H$ 4.5×10^{-5} in electrophoretic study.²⁶

¹⁷ H. M. Evans, N. Uyei, Q. R. Bartz, and M. E. Simpson, *Endocrinology*, 1938, **22**, 483.

¹⁸ L. H. Levie, *Acta brev. neerl. Physiol.*, 1937, **7**, 119; J. Freud and L. H. Levie, *Arch. int. Pharmacodyn.*, 1938, **59**, 232; J. Freud, L. H. Levie, and D. B. Kroon, *J. Endocrinol.*, 1939, **1**, 56.

¹⁹ L. H. Levie, I. E. Uyldert, and E. Dingemannse, *Acta brev. neerl. Physiol.*, 1939, **9**, 50.

²⁰ H. M. Evans, M. E. Simpson, and R. I. Pencharz, *Endocrinology*, 1939, **25**, 175; D. L. Meamber, H. L. Fraenkel-Conrat, M. E. Simpson, and H. M. Evans, *Science*, 1939, **90**, 19; A. E. Light, E. J. de Beer, and C. A. Cook, *Proc. Soc. Exp. Biol. Med.*, 1940, **44**, 189.

²¹ E. Dingemannse, *Proc. XVI Intern. Physiol. Congr.*, Zürich, 1938.

²² S. J. Folley, *Biol. Rev.*, 1940, **15**, 421.

²³ A. J. Bergmann, J. Meites, and C. W. Turner, *Endocrinology*, 1940, **26**, 716.

²⁴ R. W. Bates and O. Riddle, *J. Pharm. Exp. Ther.*, 1935, **55**, 365; *J. Biol. Chem.*, 1938, **123**, proc. v; A. White, H. R. Catchpole, and C. N. H. Long, *Science*, 1937, **86**, 82; W. R. Lyons, *Proc. Soc. Exp. Biol. Med.*, 1937, **35**, 645.

²⁵ A. White and G. I. Levin, *J. Biol. Chem.*, 1940, **132**, 717.

²⁶ C. H. Li, W. R. Lyons, and H. M. Evans, *Science*, 1939, **90**, 622; *J. Gen. Physiol.*, 1940, **23**, 433.

The crop-stimulating activity of prolactin depends upon the presence of free amino-groups in the molecule, as shown by its inactivation with nitrous acid,²⁷ with phenyl isocyanate,²⁸ and keten,²⁹ and depends upon the integrity of the tyrosine component, as indicated by its inactivation by iodine.³⁰

Anterior Pituitary Hormones and Carbohydrate Metabolism.—The information regarding this very complicated question is at present somewhat confused and contradictory; it is nevertheless evident that pituitary extracts exert a profound influence upon metabolic processes in general (respiratory-quotient reducing substances³¹) and carbohydrate metabolism in particular. It is not certain, however, to what extent these effects can be attributed to specific hormones rather than to already known active principles.

Hypophysectomised animals tend to pass into hypoglycæmia. The glycotropic (glycostatic) pituitary principle, which counteracts this tendency, is claimed to be identical with the adrenocorticotrophic principle.³² Certain pituitary extracts, named "diabetogenic," produce hyperglycæmia, sometimes ketonemia, and even permanent diabetes in dogs.³³ F. G. Young states that only globulin and ψ -globulin fractions have diabetogenic activity.³⁴

Antihormones.—The appearance in the blood of principles antagonistic to certain hormones after pretreatment with the respective hormones seems to be established³⁵ beyond doubt. Whether these substances are hormones or antibodies cannot be answered with certainty as yet.

E. K.

5. PROTEINS.

The great progress made during the last decade in our knowledge of proteins is mainly due to the application of physical methods and

²⁷ C. H. Li, W. R. Lyons, M. E. Simpson, and H. M. Evans, *Science*, 1939, **90**, 376.

²⁸ A. C. Bottomley and S. J. Folley, *Nature*, 1940, **145**, 304.

²⁹ C. H. Li, M. E. Simpson, and H. M. Evans, *Science*, 1939, **90**, 140.

³⁰ C. H. Li, W. R. Lyons, M. E. Simpson, and H. M. Evans, *ibid.*, 1940, **91**, 530.

³¹ H. M. Evans, J. M. Luck, R. J. Pencharz, and H. C. Stoner, *Amer. J. Physiol.*, 1938, **122**, 533; J. D. Greaves, J. K. Freiberg, and H. E. Johns, *J. Biol. Chem.*, 1940, **133**, 243; W. W. Billingsley, D. K. O'Donovan, and J. B. Collip, *Endocrinology*, 1939, **24**, 63.

³² J. F. Grattan and H. Jensen, *J. Biol. Chem.*, 1940, **135**, 511; *Amer. J. Physiol.*, 1940, **128**, 270.

³³ J. Campbell, H. C. Keenan, and C. H. Best, *Amer. J. Physiol.*, 1939, **126**, 455; F. G. Young, *Brit. Med. J.*, 1939, **2**, 393; C. H. Best, J. Campbell, and R. E. Haist, *J. Physiol.*, 1939, **97**, 200.

³⁴ *J. Endocrinol.*, 1939, **1**, 339.

³⁵ J. B. Collip, H. Seyle, and D. L. Thomson, *Biol. Rev.*, 1940, **15**, 1.

it is deemed desirable to review recent advances with a special emphasis on physicochemical work.

Molecular Size and Homogeneity.

Mainly as a result of the work of the Svedberg school, which has been reviewed in an earlier report,¹ we know that most of the proteins exist in solution as molecules of a well-defined size and that most of the usual protein preparations contain only one or few molecular species. The earlier chemical methods which claimed to isolate definite chemical individuals by making use of differences in solubility of proteins in salt solutions have thus been justified to a large extent. During the last few years, however, it has become increasingly clearer that the fact that a single sharp boundary is obtained in the ultra-centrifuge is not infallible evidence for the homogeneity of the preparation.² A sharp boundary, especially if it has been observed by the refractive index method, certainly shows that all the protein molecules in the solution have similar molecular weights, provided that the shapes of the molecules are also similar and are not too asymmetrical. But the fact that the molecular weights of most proteins tend to assume values which are multiples of 17,600 produces a certain *a priori* probability that a mixture of proteins will contain molecules of very similar size and thus give a fortuitous appearance of homogeneity. In fact, several such examples can be quoted. Crystalline egg albumin, which had been found to be homogeneous by sedimentation methods, has now been shown by the use of the cataphoresis method to contain two components.^{3, 4} But even electrophoretic measurements, which, when performed at different acidities, furnish one of the most reliable criteria of purity, may fail to show inhomogeneities detectable by other means. Thus R. A. Kekwick⁵ was able to separate serum albumin by fractional crystallisation into two crystalline and apparently homogeneous fractions, named A and B. The two fractions had the same electrophoretic mobilities between p_{H} 4.0 and 5.5 and identical diffusion and sedimentation constants. Fraction A contained 1.95% of carbohydrate, whereas B was almost carbohydrate-free. Similarly, several egg albumins obtained from different species of birds, which can be easily distinguished by serological reactions, show very close resemblance in electrophoresis

¹ *Ann. Reports*, 1937, **34**, 302.

² N. W. Pirie, *Biol. Rev.*, 1940, **15**, 377.

³ A. Tiselius and I. B. Eriksson-Quensel, *Biochem. J.*, 1939, **33**, 1752.

⁴ L. G. Longsworth, R. K. Cannan, and D. A. MacInnes, *J. Amer. Chem. Soc.*, 1940, **62**, 2580.

⁵ *Biochem. J.*, 1938, **32**, 552.

experiments.⁶ It has also to be appreciated that crystallisation, which is still one of the most important methods in the purification of proteins, is not a reliable indication of purity and as a criterion of homogeneity is definitely inferior to other physical or biological methods.²

Another point of importance has emerged clearly from recent work on sedimentation and electrophoresis of proteins. Although for many proteins molecular size is independent of changes in the composition of the solution within rather wide limits, for other proteins this is not the case. Syedberg had already observed in his earlier work that haemocyanin dissociates reversibly into smaller components if the p_{H} of the solution is outside the "stability range." But it has now been shown that a salt concentration of a molarity of 0.5–1.0 causes horse CO-haemoglobin to dissociate into smaller molecules.⁷ Similar effects are obtained on dilution. Bivalent ions seem to have a marked effect on molecular size even in small concentrations. Thus, the sedimentation constant of caseinogen showed an increase from 6×10^{-13} to 10×10^{-13} on addition of increasing amounts of calcium.⁸ One of the globulin components of serum, β -globulin, is particularly sensitive to changes in salt concentration.⁹ Other low-molecular substances such as urea, lysine, and ammonium chloride cause dissociation of some proteins and not of others. There is also a large amount of evidence of an interaction between different proteins. It was shown by A. S. McFarlane¹⁰ that the sedimentation diagram of a mixture of serum albumin and globulin is not an additive pattern of the isolated proteins. Pedersen⁹ has studied such interactions in a large number of cases and found that the effect varied with different proteins, and he considered that the protein-bound carbohydrate may play a part in these reactions. Electrophoretic experiments,⁴ too, indicate a protein–protein interaction in the case of egg-white.

Serum Proteins.—These changes produced in the size and shape of proteins by variations in their molecular environment assume particular importance in connection with the question, how far proteins isolated from cells and biological fluids correspond to definite chemical entities in their natural state. Serum, one of the best-studied protein systems, is a case in point. The sedimentation diagram of native untreated horse serum shows in the ultra-centrifuge

⁶ K. Landsteiner, L. G. Longsworth, and J. van der Scheer, *Science*, 1938, **88**, 83.

⁷ "The Ultracentrifuge," by The Svedberg and K. O. Pedersen, Oxford, 1940.

⁸ F. J. Philpot and J. St. L. Philpot, *Proc. Roy. Soc.*, 1939, **B**, *127*, 21.

⁹ M. Jersild and K. O. Pedersen, *Acta path. microbiol. Scand.*, 1938, **15**, 426.

fuge two main peaks.¹⁰ A lighter fraction, named "albumin," represents nearly 80% and the heavier "globulin" about 20% of the total protein. Chemical separation by half saturation with ammonium sulphate gives a ratio of albumin to globulin of nearly 1, and the same ratio is approached from the sedimentation diagram if the serum is suitably diluted. Thus the "albumin" peak can be resolved into two components, of which one is due to albumin proper and the other one to a protein called X by McFarlane and later shown by Pedersen^{7,9} to be identical with a globulin, named β -globulin by A. Tiselius. The variability of the sedimentation of this protein is caused by its extreme sensitivity to changes in the salt concentration of the medium. More important information concerning the complex system of proteins in serum and plasma was, however, obtained by the elegant electrophoretic method of Tiselius. It was shown that serum contains four components which could be distinguished by their different mobilities in an electrical field; in addition to serum albumin there are three different globulins, labelled α , β , and γ ,¹¹ having different mobilities but nearly identical molecular weights. More recently I. A. Luetscher¹² has demonstrated in human serum two albumin peaks at p_{H} 4·0, and a similar observation was made for mouse serum.¹³ By improved optical methods it was shown that the β -component of human serum is actually composed of two substances, labelled β_1 and β_2 .^{14, 15}

The values for the relative amounts of the various components in normal human serum given by different workers vary slightly, the average value for albumin being about 65%, which gives an albumin-globulin ratio of 2 : 1; the α -globulin is present in only small amounts. Plasma, as might be expected, contains another electrophoretically well-defined protein, fibrinogen.¹⁶

It is extremely difficult to correlate these electrochemical investigations, which study the protein complex of serum in its native state, with the results of workers who isolate proteins by chemical methods, such as salting-out or fractional crystallisation. It is certain that the classification of serum proteins on the basis of these old methods cannot now be accepted. It has been shown, e.g., that "pseudoglobulin" and "euglobulin" are mixtures and that "albumin" as usually prepared contains in fact a large proportion of globulin.¹⁷

¹⁰ *Biochem. J.*, 1935, **29**, 407.

¹¹ A. Tiselius, *ibid.*, 1937, **31**, 313, 1464; R. A. Kekwick, *ibid.*, 1939, **33**, 1122.

¹² *J. Clinical Invest.*, 1940, **19**, 313.

¹³ J. Bourdillon and E. H. Lenette, *J. Exp. Med.*, 1940, **72**, 11.

¹⁴ H. I. Svensson, *Kolloid-Z.*, 1939, **87**, 181.

¹⁵ R. A. Kekwick, *Biochem. J.*, 1940, **34**, 1248.

¹⁶ E. Stenhammar, *ibid.*, 1938, **32**, 719.

¹⁷ L. F. Hewitt, *ibid.*, 1936, **30**, 2229; 1938, **32**, 26.

This does not mean that fractionation by chemical methods is useless ; on the contrary, the Reporter believes that important results by such methods can still be obtained if the separation is followed by physical methods, especially by electrophoretic measurements. A very good example for such a controlled separation is Kekwick's isolation of β -globulin by fractional precipitation with sodium sulphate.¹⁵

These requirements are not fulfilled in many recent papers dealing with serum proteins ; it seems desirable, however, to review the rather complicated position at the present juncture. It was the merit of L. F. Hewitt¹⁷ to reopen the problem of the homogeneity of serum albumin and he was able to isolate three main fractions : (1) a crystalline fraction, named crystalalbumin, with practically no carbohydrate, $[\alpha_D] = 71^\circ$, high tyrosine and cystine content, and a content of tryptophan of 0·26% ; (2) an amorphous, carbohydrate-rich fraction, seroglycoid, with $[\alpha_D] = 57^\circ$, low cystine content and a tryptophan content of 1% ; (3) a fraction, globoglycoid, behaving like a globulin after crystalalbumin has been removed and containing carbohydrate. Hewitt's findings have recently been criticised by C. Rimington,¹⁸ who has also prepared a protein very rich in carbohydrate from ox-serum and named it seromucoid. There is general agreement that a crystalline fraction can be prepared from crude albumin which is carbohydrate-free ; this fraction is probably identical with Kekwick's serum albumin B.⁵ But even this preparation is not homogeneous, as two boundaries of p_H 4·0 were demonstrated by electrophoretic experiment.¹⁹ An apparently homogeneous fraction was, however, obtained by crystallising the albumin sulphate at p_H 4·0 from water.²⁰

The albumin fraction contains proteins with a high content of carbohydrate which have not been examined by physical methods ; it seems very likely that the preparations called "seromucoid" by Rimington and "seroglycoid" by Hewitt are very similar and differ only in their respective "impurities." Whether Kekwick's crystalline serum albumin A is a chemical individual or a complex formed between crystalalbumin and seromucoid remains doubtful. It seems established that the albumin fraction contains an appreciable quantity of globulin-like material ; but whether a new name should be coined for this rather ill-defined preparation is less certain. From a broader point of view the most important question in this connection is, whether serum contains a small number of distinct proteins which are in every respect chemical individuals and in which the

¹⁸ *Biochem. J.*, 1940, **34**, 931 ; C. Rimington and M. van den Ende, *ibid.*, p. 941.

¹⁹ J. A. Luetscher, *J. Amer. Chem. Soc.*, 1939, **61**, 2888.

²⁰ T. L. McMeekin, *ibid.*, p. 2884.

difficulties of isolation are due to a lack of specificity of the analytical methods used and the interaction of proteins with each other discussed above; or whether the proteins of serum contain an indefinite number of molecular species which fall into groups of certain physical and chemical similarity—a fact which would be responsible for an apparent and deceptive uniformity in physical and biological behaviour of different fractions. Physical experiments, such as electrophoresis, seem to favour the first, non-defeatist theory.

Changes in Serum Proteins in Disease.

The examination of pathological sera by modern physical methods is a very promising field, opened by A. S. McFarlane,²¹ using ultracentrifugal methods. More recently the cataphoresis technique has been applied and some interesting results have been obtained. The most striking changes occur in multiple myelomatosis, which had been known for some time to be associated with a hyperproteinemia and a change in the albumin-globulin ratio. From the results of different workers (Kekwick;¹⁵ Jersild and Pedersen;⁹ L. G. Longsworth, Th. Shedlovsky, and D. A. MacInnes²²) it appears that in all cases the relative proportion of globulin is very much increased. In one group of cases this is due to an increase in the γ -globulin peak; in others an excess of β -globulin was apparent. Nephrosis leads also to a change in β -globulin which is associated with the presence of a protein-lipoid complex; this combination is apparently broken down by ether extraction.²³ Febrile patients, on the other hand, show an increase in α -globulin.²²

Shape of Protein Molecules.

Whereas the size of proteins can be determined by methods which rest on a secure thermodynamic basis such as sedimentation equilibrium measurements, exact information as to the shape of the molecules is more difficult to obtain. Broadly speaking, proteins other than fibrous proteins can be divided into two classes. The first group comprises highly asymmetrical types such as myosin and many virus nucleoproteins which show double refraction of flow at comparatively small velocity gradients and "anomalous viscosity"; i.e., the viscosity of their solutions depends markedly on the rate of flow. The second group is made up of the so-called "globular" proteins, which do not show any definite orientation if the usual rates of flow are applied. These molecules are either spherical or do not deviate much from a symmetrical shape.

The methods most extensively used to estimate the degree of

²¹ *Biochem. J.*, 1935, **29**, 1175. ²² *J. Exp. Med.*, 1939, **70**, 399.

²³ L. G. Longsworth and D. A. MacInnes, *ibid.*, 1940, **71**, 77.

asymmetry of "globular" proteins are based either on a comparison between observed and calculated diffusion or frictional constants or on viscosity measurements. The "observed" frictional constant, f , can be calculated from the diffusion constant or from the molecular weight, M , partial specific volume and sedimentation constant; the two methods give generally identical results. On the other hand, a "theoretical" frictional constant, f_0 , can be calculated if it is assumed that the particle of the molecular weight M is rigid, spherical and does not combine with the solvent. In most cases f and f_0 are not identical and the ratio f/f_0 , which is called the frictional ratio, is greater than 1. This indicates that the particles either are solvated or are not spherical. Attempts have been made to assess the dimensions of protein molecules in solution by assuming that hydration can be neglected and that the shapes of the particles are those of ellipsoids of rotation.²⁴ These assumptions are, however, to a large extent arbitrary. Hydration cannot be neglected and may account in many cases for the values of the frictional ratio. Thus G. S. Adair²⁵ has shown that the observed diffusion constants of egg albumin, haemoglobin, and serum albumin can be quantitatively explained in terms of spherical shapes, if it is assumed that these molecules have a symmetrical shell of hydrated solvent equal in amount to that found for their crystals. It will be difficult to correct for hydration in all cases; our knowledge of the forces operating in hydration and their magnitude is still incomplete and different methods have yielded different results. Moreover, it is quite possible that, if hydration is largely due to electrostatic interaction of charged groups of the protein surface with the dipolar solvent molecules, asymmetrical hydration will take place with symmetrical particles provided that the charge density is asymmetrical. In that case the increase of the frictional ratio will be greater than could be accounted for by mere increase in volume. But, even if solvation could be neglected, the use of an ellipsoidal model is somewhat arbitrary; any marked irregularity of the molecular surface will cause an increase in f .

It is generally accepted that there exists a close relationship between the shape of molecules and the viscosity of their solutions, although the quantitative significance of the different calculations is less certain. W. Kuhn²⁶ has given an equation correlating the observed specific viscosities of solutions containing molecules of the shape of long cylinders with their axial ratios and J. M. Burgers²⁷

²⁴ H. Neurath, *J. Amer. Chem. Soc.*, 1939, **61**, 1841.

²⁵ *Proc. Roy. Soc.*, 1939, **B**, **127**, 18.

²⁶ *Z. physikal. Chem.*, 1932, **A**, **161**, 1.

²⁷ Second report on viscosity and plasticity, Amsterdam, 1938.

made similar calculations for elongated ellipsoids; A. Polson²⁸ proposed a semi-empirical formula which has given quite satisfactory results. The last equation can be considered to be a modification of Kuhn's formula, including, however, a correction for hydration. In all these calculations it is assumed that the Brownian movement is sufficient to suppress any orientation of the particles due to the hydrodynamic force applied. This assumption is almost certainly justified if the specific viscosity is independent of the rate of shear, that is, for most "globular" proteins. As pointed out by J. R. Robinson,²⁹ the position becomes more complicated in the case of highly asymmetrical particles, like tobacco mosaic virus or myosin; here work has to be done to rotate the molecules and this amount of energy becomes less as the velocity gradient is increased, compared with the energy required to maintain the rate of flow. Measurements with ordinary capillary viscometers are in such cases open to grave objections. These equations have been used by several authors to deduce the shapes of globular proteins,^{28, 30, 31} and the results obtained would indicate that nearly all the proteins which had hitherto been considered spherical have axial ratios of 1 : 3—1 : 8. These figures must, however, be accepted with some reserve. The difficulties concerning the uncertainty of hydration and shape of the particles, mentioned in connection with the frictional ratios, apply equally to the interpretation of viscosity measurements. J. W. Mehl, J. L. Oncley, and R. Simha³² have compared the values obtained from frictional ratios and from viscosity measurements and found that agreement between these methods is not satisfactory if the equations of Kuhn and Burgers are applied. An equation derived by Simha³³ gives better results. The whole problem of viscosity and shape has also recently been reviewed by J. M. Burgers,³⁴ who calculated theoretical sedimentation constants from the axial ratios obtained by viscosity measurements, assuming ellipsoidal shape, and compared these values with those observed in sedimentation experiments. The agreement was not very good, even if allowance was made for hydration. It is felt that, although the quantitative interpretation of results obtained by viscosity measurements is doubtful, they may yield important information. Thus the increase of the viscosity of egg albumin³¹ on denaturation cannot be explained by increased hydration and must be due to an increased asymmetry of shape.

²⁸ *Kolloid-Z.*, 1939, **88**, 51.

²⁹ *Proc. Roy. Soc., 1939, A, 170*, 519.

³⁰ H. Neurath and G. R. Cooper, *J. Amer. Chem. Soc.*, 1940, **62**, 2248.

³¹ H. B. Bull, *J. Biol. Chem.*, 1940, **133**, 39.

³² *Science*, 1940, **92**, 132.

³³ *J. Physical Chem.*, 1940, **44**, 25.

³⁴ *Proc. K. Akad. Wetensch. Amsterdam*, 1940, **43**, 307.

Interesting observations have also been recently reported on anomalous viscosities of proteins obtained from developing eggs and embryos.³⁵ The results, which were obtained by the use of a Couette viscometer, indicate that these proteins are highly asymmetrical.

The most reliable criterion of a marked asymmetry of shape is double refraction of flow. By the use of this method it was shown that myosin molecules are long and rod-shaped particles,³⁶ an interpretation which is in accordance with all other physical properties of this protein. Later it was shown by several workers that many other proteins show double refraction of flow at low velocity gradients. Thus a highly asymmetrical shape was proved for tobacco mosaic virus,³⁷ other plant viruses,^{38, 41} fibrinogen,³⁹ antibody globulins,⁴⁰ hog thyreoglobulin, different haemocyanins,⁴¹ and ovoglobulin.⁴² The quantitative aspects of the relationship between double refraction of flow and the dimensions of particles have recently been discussed by J. W. Mehl,⁴³ who, using the theoretical treatment of P. Boeder⁴⁴ and W. Kuhn,²⁶ calculated the length of the myosin molecule to be about 8500 Å. Such calculations are based on certain simplifications, as Mehl himself points out, and the calculated dimensions cannot be considered extremely accurate.

The influence of different chemical and physical factors on the shapes of these highly asymmetrical particles has recently been investigated. It was found, e.g., that the double refraction of flow of myosin is abolished by such substances as the chlorides of bivalent cations, guanidinium salts, and potassium iodide in very dilute solution,⁴⁵ and urea produced a similar effect in more concentrated solution. The loss of double refraction of flow was associated with a decrease of viscosity, indicating that "denaturation" in this case consisted in a definite decrease of the asymmetry of the molecule; as mentioned above, globular proteins show the opposite behaviour.

The recently developed electron microscope has also been used to

³⁵ A. S. C. Lawrence, J. Needham, and Shih-Chang Shen, *Nature*, 1940, **146**, 104.

³⁶ A. L. von Murralt and J. T. Edsall, *J. Biol. Chem.*, 1930, **89**, 315, 351.

³⁷ F. C. Bawden, N. W. Pirie, J. D. Bernal, and I. Fankuchen, *Nature*, 1936, **138**, 1951; M. A. Lauffer, *J. Physical Chem.*, 1938, **42**, 935.

³⁸ G. A. Kausche, H. Guggisberg, and A. Wissler, *Naturw.*, 1939, **27**, 303; H. S. Loring, *J. Biol. Chem.*, 1938, **126**, 455.

³⁹ G. Boehm and R. Signer, *Klin. Woch.*, 1932, **11**, 599.

⁴⁰ E. A. Kabat, *J. Exp. Med.*, 1939, **69**, 103.

⁴¹ M. A. Lauffer and W. M. Stanley, *J. Biol. Chem.*, 1938, **123**, 507.

⁴² G. Boehm and R. Signer, *Helv. Chim. Acta*, 1931, **14**, 1370.

⁴³ *Cold Spring Harbor Symposia Quantitat. Biol.*, 1938, **6**, 218.

⁴⁴ *Z. Physik*, 1932, **75**, 258.

⁴⁵ J. T. Edsall and J. W. Mehl, *J. Biol. Chem.*, 1940, **133**, 409.

observe directly the shapes of colloidal particles. Thus M. von Ardenne⁴⁶ has recently published photographs of spherical *Helix* haemocyanin molecules and two different viruses which appear in the form of long threads. Photographs of tobacco mosaic virus particles obtained by an electron diffraction method have also been published by G. A. Kausche, E. Pfankuch, and H. Ruska,⁴⁷ who state that these particles are about 150 Å. in cross-section and about 3000 Å. in length. The dimensions obtained were of the same order of magnitude as those obtained by other methods, such as viscosity. The electron microscope has also been used to study the reaction between colloidal gold particles and tobacco mosaic virus.⁴⁸ These photographs were obtained on dried films, and the shapes and sizes observed are therefore not necessarily those of native proteins or viruses.

A. N.

6. PHYSICOCHEMICAL PHENOMENA.

Metalloporphyrins.

W. M. Clark and his colleagues¹ have made an extensive survey of the use of oxidation-reduction potentials and spectrophotometry in the analysis of the behaviour of metalloporphyrins in the presence of substances capable of co-ordinating with the metal atom. Equations are obtained relating electrode potential, total concentration of metalloporphyrin, total concentration of base co-ordinating with the metal atom, the ratio of the concentrations of the oxidised and the reduced complex, the number of electrochemical equivalents involved in the oxidation-reduction process, constants describing the dissociation of the base metalloporphyrin complexes (both the reduced complex and the oxidised complex), the numbers of molecules of base co-ordinating with the metal atom (*a*) when reduced, (*b*) when oxidised, and the degree of association of the oxidised and the reduced metalloporphyrin molecules. Equations are also given by which the various equilibrium constants may be derived from spectrophotometric data. Sixteen propositions are given which are open to experimental investigation, and graphical methods are outlined for determining dissociation constants, etc., from potential measurements. Thus the analysis of these results is placed on an objective basis. This work has not so far led to many fundamentally

⁴⁶ *Naturwiss.*, 1940, **28**, 113.

⁴⁷ *Ibid.*, 1939, **27**, 292.

⁴⁸ G. A. Kausche and H. Ruska, *Kolloid-Z.*, 1939, **89**, 21.

¹ W. M. Clark, J. F. Taylor, T. H. Davies, and C. S. Vestling, *J. Biol. Chem.*, 1940, **135**, 543; J. F. Taylor, *ibid.*, p. 569; T. H. Davies, *ibid.*, p. 597; C. S. Vestling, *ibid.*, p. 623; W. M. Clark and M. E. Perkins, *ibid.*, p. 643.

new conclusions, but it makes possible the drawing of objective conclusions on many hitherto controversial points.

Protein Adsorption and the Suspended Fat of the Blood.

A. C. Frazer and his colleagues have investigated the factors responsible for the stability of the suspended plasma neutral fat. According to Frazer² the fat is mainly contained in the chylomicrons, which are microscopic or submicroscopic fatty droplets protected by an adsorbed layer of protein. Towards precipitants such as ammonium sulphate, chylomicrons behave like globulins, but when centrifuged the chylomicrons move in the centripetal direction, unlike the serum globulins. Similar behaviour is shown by artificial fat emulsions in the presence of globulins. Albumins also protect the artificial emulsions, but the precipitation reaction differs from that of chylomicrons and fat globules in the presence of globulin. Frazer concludes that the chylomicrons consist mainly of neutral fat, but that the outer layer which controls the precipitation reactions is adsorbed globulin. A. Tiselius³ has shown that the opalescence of normal serum, which is probably due to fat droplets, migrates on electrophoresis as though the particles were coated with β -globulin, and L. G. Longsworth and D. A. McInnes⁴ have shown that in serum from cases of lipoid nephrosis the lipoid behaves electrophoretically like β -globulin, after cold ether extraction much of it is removed from the serum, and the β -globulin peak of the electrophoretic pattern is correspondingly reduced.

Protein adsorption at oil-water interfaces has been studied by many authors,⁵ with conclusions compatible with those of Frazer. The first layer of protein adsorbed is denatured, as at the air-water interface, but on this layer a second layer of globular protein may be adsorbed and this layer will control the precipitation and electrophoretic properties of the interface. The loss of toxicity of toxins and venoms after mixing with oil emulsions⁶ is probably due mainly to the denaturation of proteins adsorbed on the oil droplets.

J. F. D.

² J. J. Elkes, A. C. Frazer, and H. C. Stewart, *J. Physiol.*, 1939, **95**, 68; A. C. Frazer and H. C. Stewart, *ibid.*, 4 P, 5 P.

³ *Kolloid-Z.*, 1937, **31**, 1464. ⁴ *J. Exp. Med.*, 1940, **71**, 77.

⁵ J. F. Danielli and E. N. Harvey, *J. Cell. Comp. Physiol.*, 1934, **5**, 483; H. Devaux, *Comp. rend.*, 1936, **202**, 1957; I. Langmuir and D. F. Waugh, *J. Gen. Physiol.*, 1938, **21**, 745; J. F. Danielli, *Cold Spring Harbor Symposia*, 1938, **6**, 190; A. E. Alexander and T. Teorell, *Trans. Faraday Soc.*, 1939, **35**, 727; F. A. Askew and J. F. Danielli, *ibid.*, 1940, **36**, 785.

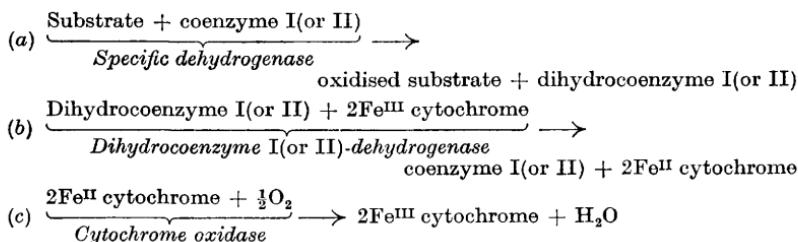
⁶ G. N. Myers, *J. Hyg.*, 1934, **34**, No. 2; A. C. Frazer and V. G. Walsh, *Brit. Med. J.*, 1934, March; J. Oerskov and S. Schmidt, *Rev. Immunol.*, 1935, **1**, No. 4; A. C. Frazer and V. G. Walsh, *J. Pharm. Exp. Ther.*, 1939, **67**, 476.

7. BIOLOGICAL CATALYSIS.

As befits the fundamental object of biochemical research, enzymic processes continue to be studied with ever-increasing revelation of their mechanisms. In particular, studies of oxidation, of C-C link disruption, and of reversible phosphorolytic breakdown of starch have yielded important results.

*Oxidation Mechanisms.**

A number of reconstructed biological oxidations can take place through three essentially similar stages, *viz.* :



(a), (b), and (c) each consist in the transfer of two hydrogen atoms (or their equivalent) from a hydrogen donor to a hydrogen acceptor under the influence of an enzyme highly specific with respect both to donor and to acceptor. Modern nomenclature tends to reserving the term "oxidase" for enzymes directly catalysing the reduction of molecular oxygen. Reactions (a) and (b) are anaerobic, whereas (c) is aerobic. It is not yet clear whether, *in vivo*, the reoxidation of dihydrocoenzymes takes place through the cytochrome system or whether some alternative mechanism is concerned. It is, however, significant that it is generally believed that cyanide, known to inhibit cytochrome oxidase, blocks the greater part of most tissue-respiration.

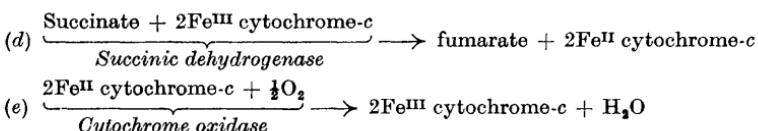
In this brief review it is convenient to regard both enzymes and carriers as catalysts. The view has recently been put forward that coenzymes I and II (loosely termed "phosphopyridine nucleotides") should not be regarded as individual catalysts, but as being dissociably linked to "specific proteins" (the dehydrogenases) and thus forming the prosthetic group of a catalytically-active conjugated protein. This idea has been suggested from the recognition of the nature of the cytochromes and of the so-called "flavoproteins" (Report for 1939, p. 353). M. Dixon and L. G. Zerfas,¹ however, have in-

¹ *Biochem. J.*, 1940, **34**, 371.

* D. E. Green, "Mechanisms of Biological Oxidations" (Cambridge Univ. Press, 1940).

geniously obtained evidence in direct contradiction to this conception and in support of the older and more widely held view. By choosing appropriate substances to act as hydrogen-acceptors, they showed that both the alcohol and the maleic dehydrogenases of yeast could oxidise their appropriate substrates in complete absence of coenzyme I. They therefore regard the "protein" of the dehydrogenases as the complete enzyme and point out that coenzyme I and the "protein" are in the relation of substrate to enzyme as indicated in (a) above.

The cytochromes and the succinic dehydrogenase system have been the subject of new investigations by D. Keilin and E. F. Hartree.² They emphasise that attempts to dissect the system (which oxidises succinate aerobically) have failed. The activity of the system depends on the presence of insoluble particles to which the catalysts are bound. The generally accepted course of succinate oxidation may be expressed as follows :



But Keilin and Hartree have found that with certain oxidase preparations reaction (d) would not proceed. They suggest that the reduction of Fe^{III} cytochrome-c may be an indirect one, requiring the intermediate intervention of cytochrome-*b* or some hitherto unrecognised substance. These authors have recognised, in heart-muscle, insect thoracic-muscle, baker's yeast, and strictly aerobic bacteria a new cytochrome component, termed a_3 . This has many properties identifying it with the enzyme, cytochrome oxidase; e.g., it is thermolabile and is, in the Fe^{II} state, very easily autoxidisable. Carbon monoxide combines with the Fe^{II} compound, giving a stabilised derivative, whereas the Fe^{III} form combines reversibly with potassium cyanide, hydrogen sulphide, sodium azide, etc. On the other hand, it has so far not been possible to demonstrate either direct or indirect reduction of $\text{Fe}^{\text{III}}a_3$ by $\text{Fe}^{\text{II}}c$ under strictly anaerobic conditions and in complete absence of other reducing substances. The original papers should be consulted.

Fission of the C-C Link.

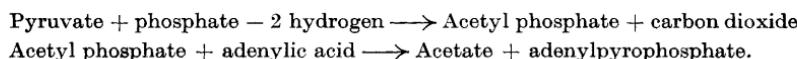
Remarkably little is known of biological processes involving the degradation of a carbon chain. Carboxylase (which decarboxylates α -keto-acids, $\text{CH}_3\text{R}_1\text{R}_2\text{CO}\cdot\text{CO}_2\text{H}$) and zymohexase (which splits

² Proc. Roy. Soc., 1939, B, 127, 167; 1940, B, 129, 277.

fructofuranose 1 : 6-diphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) are probably the only authentic members of this class to be identified as individuals.

Pyruvate is known to undergo two different degradation processes *in vivo*. In yeast, carboxylase in the presence of thiamine (aneurin) diphosphate, forms acetaldehyde and carbon dioxide from pyruvate. In animal tissues, and in certain bacteria, pyruvate is oxidised to acetate and carbon dioxide, thiamine diphosphate again being necessary. D. E. Green, D. Herbert, and V. Subrahmanyam³ describe the isolation from brewer's yeast of a highly active, stable preparation of carboxylase containing 0·46% of thiamine diphosphate and 0·13% of magnesium, no other metal being detected. The authors regard their material as containing the complete enzyme. In high concentrations of salts the three components, protein, magnesium, and thiamine diphosphate, are bound together; on dilution, dissociation takes place. Other bivalent cations can replace magnesium; the following were found active in descending order : Mn, Mg, Fe^{II}, Cu, Cd, Zn.

The oxidative fission of pyruvate by preparations of *Bact. delbrückii* has been studied by F. Lipmann.⁴ The original papers should be studied for details; for present purposes it will suffice to discuss Lipmann's views on the part played by phosphate in this reaction. Recognising the importance of *enolpyruvic acid* phosphate in glycolysis (Report for 1939, p. 358), he sought for possible phosphorylated intermediaries in the oxidation. He found (i) that phosphate was transferred to adenylic acid, and (ii) that crude acetyl phosphate⁵ in the presence of the bacterial material could serve as a source of phosphate for reaction (i). Lipmann has therefore suggested the following scheme for this oxidation :



It should be noted that S. Ochoa, R. A. Peters, and L. A. Stocken^{5a} report that acetyl phosphate does not act as an intermediary in pyruvate oxidation by brain nor does it act as a phosphate donor to adenylic acid in muscle extract.

The widely distributed zymohexase (Report for 1939, p. 359) has been obtained as a very active preparation by D. Herbert, H. Gordon, and V. Subrahmanyam⁶ from the water-soluble protein of

³ *J. Biol. Chem.*, 1940, **135**, 795.

⁴ *Nature*, 1939, **144**, 381; *Cold Spring Harbor Symposium on Quantitative Biology*, 1939, **7**, 248; *J. Biol. Chem.*, 1940, **134**, 436.

⁵ E. Kamerer and G. Carius, *Annalen*, 1864, **131**, 165.

^{5a} *Nature*, 1939, **144**, 750. ⁶ *Biochem. J.*, 1940, **34**, 1108.

rabbit muscle. Some 4% of this fraction was isolated with an activity 150 times greater than that of the original tissue. The preparation was non-crystalline and contained C, 50.6; H, 7.04; N, 15.8; S, 1.25%; neither phosphorus nor iodine nor significant amounts of carbohydrate were detected. An exhaustive study of the enzyme was made; it was concluded that no oxidisable or reducible group was concerned in its activity; heavy metals had a marked inhibitory effect. The material was examined by E. C. Bate Smith in the Tiselius apparatus.

The Reversible Phosphorolysis of Starch : Phosphorylation of Glucose.

In the Report for 1939 (pp. 359, 361) mention was made of the reversible splitting of glycogens from liver, muscle, and yeast into glucopyranose 1-phosphate. Two papers by C. S. Hanes⁷ describe the extension of this work to starch, enzymic preparations having been obtained from both pea-seeds and potatoes (see this vol., pp. 419, 421). In such systems the normal course undergone by the glucose 1-phosphate is its conversion into hexose-6-phosphates. Only if the latter reaction can be prevented does the 1-phosphate accumulate. In that event synthesis of the polysaccharide tends to result, as the enzymic equilibrium is on the side of synthesis. It would therefore be of interest to demonstrate the production by enzymes of the 1-phosphate from glucose and $H_2PO_4^-$. This has not yet been done.

Although yeast preparations are believed to esterify carbohydrate with phosphoric acid, it is only recently that the phosphorylation of glucose by animal tissues has been demonstrated, although only with respect to kidney extracts. H. Kalckar⁸ has shown that phosphoric esters accumulate in such extracts, under aerobic conditions, when fluoride is added to inhibit phosphatase activity. The process is stimulated by the presence of alanine, glutamic, citric, and fumaric acids, all of which can be oxidised by the kidney. Later, S. P. Colowick, M. S. Welch, and C. F. Cori⁹ showed that fructose diphosphate and glyceric acid phosphate are formed in aerobic kidney extracts in the presence of fluoride. The authors consider that the phosphorylating activity is effected through energy derived from the oxidation processes involving dicarboxylates, e.g., succinate to fumarate. Adenylic acid, coenzyme I and Mg⁺⁺ are necessary. The same authors¹⁰ have further shown that, in the absence of fluoride, added fumarate catalyses the oxidation both of glucose and of

⁷ Proc. Roy. Soc., 1940, B, 128, 421; 129, 174.

⁸ Enzymologia, 1939, 6, 209.

⁹ J. Biol. Chem., 1940, 133, 359.

¹⁰ Ibid., p. 641.

pyruvate by dialysed kidney extract. They therefore consider that fumarate is an essential link between the phosphorylation of glucose and its subsequent oxidation.

Carbonic Anhydrase and Zinc.

D. Keilin and T. Mann¹¹ have isolated extremely active protein preparations from erythrocytes and gastric mucosa. The material is remarkable in containing zinc, which appears to be necessary for its activity. It has further been shown¹² that sulphanilamide, and in general, sulphonamides, act as specific inhibitors for carbonic anhydrase.

Crystalline Preparations of Enzymes.

The following list gives those enzymes which to date have been obtained in the form of crystalline protein preparations. (For a discussion on the problem of the homogeneity of crystalline proteins, see this vol., p. 405.)

Pepsin,¹³ pepsinogen, trypsin, trypsinogen, chymotrypsin, chymotrypsinogen, papain,¹⁴ carboxypeptidase, nuclease,¹⁵ urease, catalase, alcohol dehydrogenase, triosephosphate dehydrogenase,¹⁶ lactic dehydrogenase of heart,¹⁷ tyrosinase.¹⁸

(References are given only to the most recent advances.)

D. J. B.

8. PLANT BIOCHEMISTRY.

Some Products and Enzymes of Plants.

Starch and Amylases.—One of the outstanding achievements of the year in the field of carbohydrate biochemistry is due to C. S. Hanes,¹ who has published interesting and valuable papers on the breakdown and synthesis of starch in the higher plants, and has been able to effect the synthesis *in vitro*. The origin of the investigations may be said to arise from an attempt to discover whether the phosphorylated sugars play a similar rôle in the carbohydrate metabolism of the higher plants, to that which they have been shown to

¹¹ *Biochem. J.*, 1940, **34**, 1163.

¹² T. Mann and D. Keilin, *Nature*, 1940, **146**, 164.

¹³ V. Desreux and R. M. Herriot, *ibid.*, 1939, **144**, 289.

¹⁴ A. K. Balls and H. Lineweaver, *J. Biol. Chem.*, 1939, **130**, 669.

¹⁵ M. Kunitz, *J. Gen. Physiol.*, 1940, **24**, 15.

¹⁶ O. Warburg and W. Christian, *Biochem. Z.*, 1939, **303**, 40.

¹⁷ F. B. Straub, *Biochem. J.*, 1940, **34**, 483.

¹⁸ H. R. Dalton and J. M. Nelson, *J. Amer. Chem. Soc.*, 1939, **61**, 2946.

¹ *Proc. Roy. Soc.*, 1940, **B**, **128**, 421.

do in the case of yeast and some animal tissues. Necessary stepping stones to the proof of such a theory are the discovery of phosphorylated sugars, and of enzymes capable of acting on them, in the plant economy. A phosphorylating enzyme had been discovered by J. Bodnar² in 1925 in the flour from ground mature peas, and B. Tankó³ in 1936 was able to show that such an enzyme converted inorganic phosphate in a phosphate buffered mixture of the flour into fructofuranose 1 : 6-diphosphate. Hexose monophosphates also were present. Hanes has found that the enzyme, termed phosphorylase, separated from the tissue, is able to phosphorylate starch and a number of starch dextrans and that simple sugars with the exception of maltose, which is attacked only slowly, are unattacked. The first product in the phosphorylation of starch is the non-reducing glucose 1-phosphate, and this is found to be a reversible reaction in that addition of glucose 1-phosphate to an extract of peas involves the production of a certain proportion of starch with liberation of free phosphate. An alternative transformation of glucose 1-phosphate involving two distinct enzymic actions has been observed: the glucose 1-phosphate rapidly disappears from the system and a mixture of glucose-, fructose-, and mannose-6-phosphate is produced. Tankó's observation that hexose diphosphate is formed when pea flour suspensions act in presence of phosphate is confirmed and it is further found that addition of starch to the mixture greatly accelerates the esterification. If dialysed extracts are used, however, the diphosphate is not formed and it appears that such formation depends on the presence of a dialysable co-enzyme.

The glucose 1-phosphate appears to be identical with that obtained as the first product of esterification in the action of muscle phosphorylase on glycogen.⁴

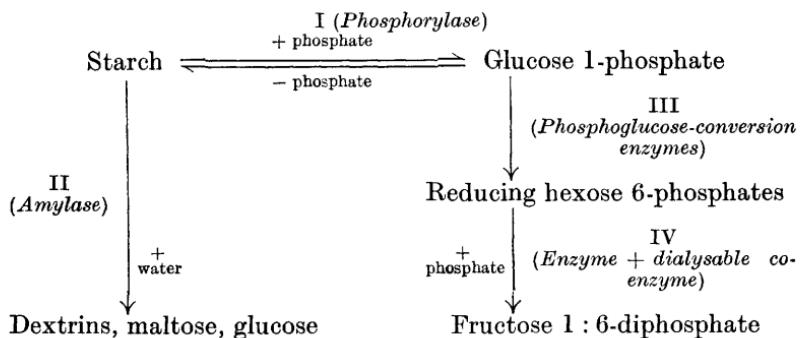
Observation shows that it is probable that the glucose 1-phosphate arises by direct phosphorylation of the saccharide chains in starch. Thus starch and complex dextrans are esterified more rapidly and completely than the lower members of the starch-maltose series; that the higher members are directly involved in the reaction is shown by the decrease in iodine colour under phosphorylase action; and the change in iodine colour corresponds with what might be anticipated from an endwise degradation of the chains. Glucose itself is not esterified and from these facts it is concluded that terminal glucose units of the chains are phosphorylated and liberated from non-aldehydic chain-ends.

² *Biochem. Z.*, 1925, **165**, 1.

³ *Biochem. J.*, 1936, **30**, 692.

⁴ C. F. Cori and G. T. Cori, *Proc. Soc. Exp. Biol. Med.*, 1936, **34**, 702.

Hanes summarises the preceding results as follows :



At a later stage, Hanes⁵ describes the preparation of phosphorylase from potato tubers; the enzyme is obtained in highly active form and is free from those enzymes which promote the alternative transformations already referred to. The reversibility of the starch-glucose 1-phosphate reaction is shown, since the ratio of inorganic phosphate to ester reaches the same value in either direction. Moreover, the equilibrium point is not affected by wide variations in concentrations of the reactants or enzyme. The position of equilibrium is notably altered by changes in p_{H} and it has been shown that this is due to the effect of varying hydrogen-ion concentration on the dissociation of inorganic and ester phosphate. The bivalent ions determine the equilibrium, and between p_{H} limits of 5 and 7 a constant value of 2.2 is found for the ratio $[\text{HPO}_4^{''}]/[\text{C}_6\text{H}_{11}\text{O}_5\cdot\text{O}\cdot\text{PO}_3^{''}]$.

Large-scale preparations of pure glucose 1-phosphate as the crystalline potassium salt are described, and by the action of purified phosphorylase on glucose 1-phosphate large amounts of a polysaccharide resembling potato starch have been obtained. The product shows the typical granules of native starch when viewed under the microscope; but further examination shows certain points of difference from natural potato starch. For example, the synthetic product is sparingly soluble in water and rapidly retrogrades when in solution; again, the iodine colour is much more intense than that given by potato starch. A third point of difference was observed in relation to its degradation by the β -amylase of ungerminated barley. Natural starches normally degrade to about 60% of maltose and the residue consists of resistant α -amylodextrin. The enzymically prepared starch, however, allows the action of the β -amylase to proceed almost to completion, 95—100% of the preparation being converted into maltose. The synthetic product corre-

⁵ Proc. Roy. Soc., 1940, B, 129, 174.

sponds thus most closely to the amyloamylose⁶ fraction of starch. Much interest thus attaches to further investigation of the enzyme mechanisms involved in these remarkable transformations and to constitutional studies on the synthetic polysaccharide, which are now proceeding.

X-Ray comparisons between the natural and the synthetic starch are discussed by W. T. Astbury, F. O. Bell, and C. S. Hanes⁷ in a recent note. The A-, B-, C-, and V-powder photographs correspond to wheat starch, potato starch, a mixture of the two, and to alcohol-precipitated starch respectively.⁸ The only difference observable between the photographs of potato starch and the synthetic starch is that the former is very slightly sharper. A further curious point was discovered in that amyloamylose precipitated by alcohol after preparation by electrophoresis gave the V-photograph, whereas the synthetic starch still gave the B-photograph after precipitation with alcohol. A re-examination of the conditions governing the production of the different types of X-ray photograph may throw further light on the relation between the various types of starch. It is not clear at present whether different photograph types are due to differences in phosphorylases or, as seems more probable, in the method of crystallisation, *i.e.*, preparation of the starches. The probability of the latter explanation is indicated by the fact that A-, B-, or C-photographs may be obtained from the same starch at different temperatures of deposition.⁹

A number of well-known factors influence the rate of breakdown of starch by enzymes and the nature of the products. It has recently been shown that comparatively mild treatment of starch prior to diastatic action leads to a much greater degradation than is possible with untreated starch. Thus, R. H. Hopkins, E. G. Stopher, and D. E. Dolby¹⁰ describe the separation of starch into amylopectin and amyloamylose by electrophoresis, the starch having been previously treated in one of two ways. In some experiments the starch was finely ground in order to rupture the granules; in others the starch was made directly into a paste and dispersed at 120°. By alternate redispersion and electrophoresis the amylopectin fraction yields high proportions of amyloamylose, and the latter is far more completely degraded by barley diastase, but at a slower rate, than the untreated starch. The conversion into amyloamylose

⁶ M. Samec, *Z. physiol. Chem.*, 1936, **236**, 103.

⁷ *Nature*, 1940, **146**, 558.

⁸ J. R. Katz, "Die Röntgenspektrographie usw.," Berlin and Wien, 1934.

⁹ J. R. Katz and J. C. Derkson, *Z. physikal. Chem.*, 1933, **A, 165**, 228.

¹⁰ *J. Inst. Brew.*, 1940, **46**, 426.

may reach yields as high as 80% if dispersion is carried out above 100°; the product reverts on standing to a substance which behaves towards diastase as does the starch from which it is obtained.

In recent years some doubt and minor controversy have arisen with reference to the effect of the particular buffer solution employed on the activity of enzymes. Not only has the nature of the buffer been held to affect the activity, but it has been suggested that ionic strength is an important factor. Experiments designed to investigate these points in the case of taka-diastase are described by G. A. Ballou and J. M. Luck.¹¹ Using a starch substrate, a temperature of 30°, and a series of buffers of an ionic strength of 0·05N, they found that the optimum p_{H} , in respect of saccharogenic action, was 5·1 for formate, acetate, propionate, butyrate, valerate, phenylacetate, and succinate buffers. A slight shift to $p_{\text{H}} 5\cdot4$ was observed for buffers containing phthalate or citrate. Where the enzyme was employed at the optimum p_{H} or at a reaction on the alkaline side of this value, variation in the anion of the buffer was without effect on the relative activity; but on the acid side of the optimum, marked differences were observed with varying anions.

Employing the usual methods of methylation and end-group assay, E. G. E. Hawkins, J. K. N. Jones, and G. T. Young¹² have found that the starch present in unripe bananas conforms structurally to the usual pattern in that the repeating unit consists of a chain of about 24 glucose residues. Similarity to rice starch¹³ is further indicated by the close resemblance of the course of disaggregation in the two cases. Disaggregation was discussed in last year's Report¹⁴ and is effected in methylated starches by hydrolysis with oxalic acid in a mixture of methyl alcohol and water. The hydrolysis takes place at the bonds between the repeating units, since, although products of lower molecular weight are obtained, the chain length of the repeating unit remains unchanged.

A more rapid method of assay of the end group in methylated starch is reported by S. Peat and J. Whetstone.¹⁵ The method involves acetolysis of methylated starch by acetyl bromide in cold chloroform. Treatment of the product with methyl alcohol gives a mixture of methyl glucosides from which the tetramethyl glucoside is separated by distillation. The removal of the end group is selective and rapid, the tetramethyl derivative separating within five minutes of the commencement of the reaction.

Pectin, Mucilages, etc.—Constitutional studies on pectic acids continue and are involving the preparation and characterisation of

¹¹ *J. Biol. Chem.*, 1940, **135**, 111.

¹² *J.*, 1940, 390.

¹³ E. L. Hirst and G. T. Young, *J.*, 1939, 1471.

¹⁴ *Ann. Reports*, 1939, **36**, 272.

¹⁵ *J.*, 1940, 276.

a number of derivatives of galacturonic acid exhibiting a fructofuranose structure. Among these may be mentioned the methyl ester of 2 : 3 : 5-trimethyl β -methylgalacturonoside, which has been synthesised by S. Luckett and F. Smith.¹⁶ In the course of investigations on citrus pectic acids, these authors¹⁷ have also isolated the methyl ester of 2 : 3-dimethyl methylgalacturonoside and have suggested that citrus pectic acid is built up of pyranose residues of galacturonic acid joined by 1 : 4 α -glycosidic linkages. Osmotic pressure measurements on the methyl ester of methylated pectic acid appear to indicate a small molecule of about 13 units.

Using such sources as Tuso pith and sliced radishes, S. Onon¹⁸ has isolated pectic substances on the usual lines and with the usual properties. A method of extraction is of interest, however, in that the extractive is boiling water containing copper sulphate. By this means it is claimed that pectins are obtained of a snow-white colour, and of a galacturonic acid and methoxyl content much higher than those normally obtained. The resulting pectin is considered to be a polymerised trimethyl tetragalacturonic acid and contains no araban or galactan residues.

Valuable contributions to the study of jelly formation by pectin have been made by C. L. Hinton,²⁰ who suggests that pectins may be regarded as complex mixtures of carboxylic acids whose constituents cannot be separated at present. However, they may be studied from a physicochemical standpoint and much information has been gained in this way. Thus the effect of variation of a number of factors, such as concentration of pectin and of other soluble substances present, the p_H of the mixture, the effect of de-esterification of pectin by pectase or alkali, on the strength of the jelly has been studied. Electrolytic dissociation of pectin was investigated and the "constant" was found to diminish as neutralisation with alkali became more complete; but there was no important change in the constant for pectins boiled for one hour or de-esterified enzymically, or by citric acid. Jelly formation involves only those molecules of pectin which are in the un-ionised condition, and these must reach a certain solubility or saturation limit varying with the total solids concentration of the mixture. The strength of a particular jelly with a given buffer salt was found to be proportional to the amount of non-ionised pectin above the solubility limit. The ratio of jelly strength to the amount of jelling substance differed for different pectins. This theory of jelly formation has been elaborated and has explained many of the phenomena observed, especially those relating to the effect of p_H . Encouraging results

¹⁶ *J.*, 1940, 1114.

¹⁷ *Ibid.*, p. 1106.

¹⁸ *Bull. Sch. Agric., Taihoku*, 1940, 1, 1.

²⁰ *Biochem. J.*, 1940, 34, 1211.

may be expected to follow from this investigation, which is probably the first attempt to treat in ordered and mathematical fashion the mass of diffuse and often contradictory data which have tended to obscure rather than clarify the problem.

The constitution of the mucilage of carrageen moss (*Chondrus crispus*) is the subject of investigation by T. Dillon and P. O'Colla,²¹ who submit the mucilage to acetolysis in the usual manner and obtain, after removal of acetyl groups, two polysaccharides which appear to be galactans. These correspond to those found by P. Haas *et al.*,²² in that one is soluble in water and one soluble only in hot water. The latter workers were unable to isolate the polysaccharides, which they showed to exist as calcium salts of ethereal sulphates. The carbohydrate hydrolysis products appeared to be chiefly galactose and fructose, but Dillon and O'Colla were unable to confirm the latter, although indications were obtained that fructose was present in solution on deacetylation.

In another communication on the same subject, E. G. V. Percival and J. Buchanan²³ confirm the work of Haas and criticise the later findings of Dillon and O'Colla on the grounds that their method of acetolysis involved considerable degradation of the polysaccharides, all constituents except galactose being lost. This indicates that galactose must be contained in the most resistant portions of the molecule, but the complexity of the latter is indicated by other hydrolysis products, which appear to include glucose, a pentose, and possibly a ketose. The probable configuration of the molecule is responsible for the difficulties observed in acetolysis and methylation.

A product of the nature of a polysaccharide and hydrolysing largely to xylose is reported for the first time from the red alga, *Rhodymenia palmata*, by V. C. Barry and T. Dillon.²⁴ The alga is immersed in dilute hydrochloric acid for 24 hours and from the resulting viscous solution a white precipitate is obtained with alcohol. The precipitate yielded crystalline xylose in hydrolysis with dilute nitric acid. A similar treatment on the case of *Dulsea edulis* produced a substance which appeared to be similar to the mucilages from other marine algae. It was an ethereal sulphate, contained no xylose, and was oxidisable to mucic acid.

Attempts have been made from time to time to isolate polysaccharides similar to naturally occurring plant gums by the action *in vitro* of bacteria, normally associated with a particular plant, on sucrose in artificial media. The work of E. A. Cooper and J. F.

²¹ *Nature*, 1940, **145**, 749.

²² *Biochem. J.*, 1921, **15**, 469; 1922, **16**, 578; 1929, **23**, 425.

²³ *Nature*, 1940, **145**, 1020.

²⁴ *Ibid.*, 1940, **146**, 620.

Preston has been recorded in these Reports²⁵ and the subject is renewed by R. R. Lyne, S. Peat, and M. Stacey,²⁶ who find that polysaccharides of the levan type are formed by *B. megatherium*, *Bact. pruni*, and *Bact. prunicola* under the above conditions. So far, polysaccharides comparable with the gums have not yet been produced. Examination of the hydrolysed methylated levans showed that each could be represented by a chain of 10—12 fructose units, and in this respect they resembled the levans produced by *B. subtilis*. Differences in properties among the levans are thought to be due to varying degrees of aggregation of the repeating unit. A practical point of some importance is the observation that the polarimetric rotation of the acetates in chloroform solution depends on the water content of the reaction mixture and this probably explains discrepancies in results by previous workers.

Hemicelluloses.—Although much analytical investigation of the cell-wall of plant tissues has been conducted, in most cases it is the mature material which has been examined, and little work by comparison has been expended on the developing tissue. Such investigation would prove of value in the formulation of theories of the origin of the constituents of mature tissue. A. Allsopp and P. Misra²⁷ have studied the common ash, the common elm and Scotch pine, whose tissue they divide into three "fractions": the cambium, together with the differentiating xylem; newly formed wood; and mature sapwood. The composition of the first-named fraction showed similarities with that published for other young tissues. A high pectin content is characteristic of the young cell wall and in this and the lower lignin : cellulose ratio the cambium and differentiating elements contrast chiefly with the mature wood. Even after vessel differentiation is complete there are small changes in composition, involving the loss of pectin, increase in encrusting pentosans and in the resistance of the lignin constituents. Theories of origin of the constituents of mature cell-walls based on the observed fact that, for instance, a fall in pectin concentration during lignification indicates that the lignin arose from the pectin, must be treated with reserve, since a change in concentration is no indication of a change in total quantity present. The same remark applies to the suggestion that hemicelluloses are derived from pectin.

Osmotic pressure and viscosity studies on a number of polyoses of wood by E. Husemann²⁸ indicate a wide range of molecular size, the particles responsible for the development of osmotic pressure being molecules and not molecular aggregates. Degree of polymerisation is of the order of 150—220 units in the case of xylans,

²⁵ *Ann. Reports*, 1938, **35**, 378.

²⁶ *J.*, 1940, 237.

²⁷ *Biochem. J.*, 1940, **34**, 1078.

²⁸ *Naturwiss.*, 1939, **27**, 595.

from wheat straw and beechwood, mannan from spruce and arabogalactan from larch. These values are small compared with that for beech cellulose, which is given as not less than 1500. The degree of polymerisation is unaltered on conversion of the products into acyl derivatives. On the basis of viscosity measurements it is thought that, whereas the mannan and xylans comprise long chains, the arabogalactan molecule consists of multi-branched chains.

M. H. O'Dwyer,²⁹ continuing her investigations on the hemicelluloses of oak wood, has found that the transition from sap-wood to heart-wood as shown by preparation of hemicellulose A from the two types involves definite constitutional changes in this component. The greater proportion of the carbohydrate residues in the molecule are anhydroxylose and these are combined with uronic acid and methoxyaldobionic acid residues, the former predominating in the hemicellulose of the sap-wood, the latter in that of the heart-wood. A further difference was also noticed, in that the hemicellulose preparations from sap-wood all gave the iodine blue coloration typical of starch, whereas the heart-wood preparations gave no iodine colour. Although at this stage glucose was not found as a hydrolysis product of the sap-wood preparations, it was suggested that the blue coloration was due to the presence of anhydroglucose residues in the hemicellulose.

In a later communication O'Dwyer³⁰ reports that hemicellulose A may be split up into two polysaccharide fractions by the action of taka-diastase and in addition some 10% of the glucose has been obtained from sap-wood hemicellulose A. The action of water at 100° effects a similar scission, but glucose is not split off. The presence of glucose is seen to be the only difference between the hemicellulose A of sap- and heart-wood,³¹ the polysaccharide fractions after prolonged hydrolysis with taka-diastase being identical. The complete hydrolysis involves production of two parts of a soluble polysaccharide and three parts of xylose. The molecule of the polysaccharide appears to consist of six anhydroxylose units combined with one methyluronic anhydride unit.

In the latest paper³² to date similar results are obtained with hemicellulose B. Again the sap-wood hemicellulose only gives the iodine coloration and contains anhydroglucose units. The products of fission under the action of taka-diastase appear to be the same for all the hemicelluloses examined. Hemicelluloses have been prepared by E. Anderson, M. Seeley, W. T. Stewart, J. C. Redd, and D. Westerbreke³³ from various hardwoods before and after chlorin-

²⁹ *Biochem. J.*, 1934, **28**, 2116.

³⁰ *Ibid.*, 1937, **31**, 254.

³¹ *Ibid.*, 1939, **33**, 713.

³² *Ibid.*, 1940, **34**, 149.

³³ *J. Biol. Chem.*, 1940, **135**, 189.

ation. Their results confirm much that has been suggested by W. G. Campbell,³⁴ M. H. O'Dwyer, and others with reference to the origin of hemicelluloses in woods. Two of the woods examined, lemon wood and black locust sap-wood, contained starch and all of the hemicelluloses from these woods gave a blue or pink coloration with iodine. These hemicelluloses appear to contain anhydroglucose groups in the xylan chain, and may possibly represent intermediate products in the transformation of starch or its degradation products into hemicelluloses. The hemicelluloses of birch wood and black locust heart-wood did not contain starch, gave no typical colour with iodine, and were differentiated from the other hemicelluloses in chemical composition, since the xylan groups were combined with a monomethylated uronic acid. The probable number of xylan groups in the chain varies with different hemicelluloses and appears to approximate to 19 in the largest molecules and 8 in the smallest. The carboxyl group of the uronic acid may be involved in attachment of the hemicellulose to some other substance of the cell-wall. This might explain the fact that, although the hemicellulose is not extractable from the wood by hot water, it is nevertheless somewhat soluble in hot water after extraction with sodium hydroxide.

Hemicelluloses prepared by the usually recognised methods are reported from a number of sources, including oat hulls,³⁵ wheat-straw,³⁶ and lucerne hay.³⁷ Those obtained from wheatstraw consist mainly of the B-fraction with small amounts of A and C. The hydrolysis products include xylose, arabinose, and possibly a methyl derivative of glucuronic acid; the xylose predominates. The same remarks apply to the hemicellulose of lucerne hay.

The methods adopted in the pre-treatment of materials employed for the preparation of hemicelluloses have given rise to some controversy, a principal bone of contention being the use of alcoholic sodium hydroxide for the removal of lignin. This method was used by F. W. Norris and I. A. Preece,³⁸ but was shown later by the latter author³⁹ to involve some loss of furfuraldehyde-yielding material. The procedure was also criticised by A. G. Norman⁴⁰ on similar grounds. Nevertheless, S. Angell and F. W. Norris⁴¹ found that in the pre-treatment of the flowers of the hop, no such loss could be observed after treatment with alcoholic soda, and they suggested that the effect of alcoholic soda depended on the material under

³⁴ *Biochem. J.*, 1935, **29**, 1068.

³⁵ P. W. Krznarich, *Cereal Chem.*, 1940, **17**, 457.

³⁶ H. D. Weike and M. Phillips, *J. Agric. Res.*, 1940, **60**, 781.

³⁷ M. Phillips and B. L. Davis, *ibid.*, p. 775.

³⁸ *Biochem. J.*, 1930, **24**, 59.

³⁹ *Ibid.*, 1931, **25**, 1304.

⁴⁰ *Ibid.*, 1935, **29**, 545; 1937, **31**, 1579.

⁴¹ *Ibid.*, 1936, **30**, 2159.

investigation. The subject is again opened by I. A. Preece,⁴² who employed teak sawdust as the raw material, and showed that there is definite loss of furfuraldehyde-yielding material when the wood is submitted to either alcoholic or aqueous soda extraction. The extracted hemicelluloses themselves were not stable under these reagents. The author concludes that pre-treatment with alcoholic soda does reduce the lignin content of subsequent preparations of hemicellulose, but the disadvantage of the treatment outweighs the advantages. The choice of extractive must depend on the material and on the purpose in view in the preparation of the hemicellulose. Alcohol extraction may be employed in some cases, or alcoholic soda treatment in the cold, as employed by H. W. Buston⁴³ and A. G. Norman.⁴⁰

Plant Proteases.—The isolation of papain in crystalline form has been achieved by A. K. Balls and H. Lineweaver,⁴⁴ who precipitated papaya latex successively with ammonium sulphate and sodium chloride at suitable concentrations and p_{H} in the presence of sodium cyanide. The crystals were usually obtained in fine needles, which changed on long standing in water to elongated hexagonal plates. Analysis showed that the enzyme was of a protein nature, containing 15.5% of nitrogen, 1.2% of total sulphur, and 1% of cysteine sulphur. A molecular weight of about 30,000 was indicated by osmotic pressure measurements and by the evidence of the ultra-centrifuge. The enzyme was only sparingly soluble in dilute saline solutions, but soluble in 70% alcohol. The isoelectric point was at p_{H} 9. The presence of an activator such as cyanide or cysteine was essential for the preparation of crystals of high activity and such activity corresponded to a maximum value per unit of protein nitrogen for all crystals, although prepared in different ways.

The presence of sulphydryl groups as necessary for papain activity is thus again indicated by the preceding work on crystalline papain. Activation-inhibition reactions of a group of similar plant proteases are investigated by D. M. Greenberg and T. Winnick,⁴⁵ who employ the bromelin of pineapples; solanain from the horse-nettle, *Solanum elaeagnifolium*; a new protease from the latex of milkweed, *Asclepias mexicana*; and a protease from another milkweed, *Asclepias speciosa*. In accordance with a recent suggestion it is proposed to use the suffix "ain" for new plant proteases, and for present purposes the last two enzymes are designated *asclepain m* and *asclepain s*. All the enzymes with the exception of solanain show reactions which suggest that they are all related to papain, the presence of a sulphydryl group being necessary for activity. Sola-

⁴² *Biochem. J.*, 1940, **34**, 251.

⁴³ *Ibid.*, 1934, **28**, 1028.

⁴⁴ *J. Biol. Chem.*, 1939, **130**, 669.

⁴⁵ *Ibid.*, 1940, **135**, 761.

nain is unaffected by oxidising or reducing agents or reagents which react with sulphhydryl groups. It is not a papainase and it is probable that phenolic groups may be essential for its activity as indicated by inactivations produced by nitrous acid and keten. There is some evidence also that the papainases may require phenolic in addition to sulphhydryl groups.

p_{H} -Activity curves for the different enzymes, obtained with denatured haemoglobin and ovalbumin as substrates, indicate an optimum p_{H} with haemoglobin of 6.5—8.5, and with ovalbumin of 7—7.5. The character of the curve has been shown to depend more on the electrochemical nature of the enzymes than on the degree of dissociation of the substrates.⁴⁶

In a third communication⁴⁷ the authors discuss the kinetics of the action of the enzymes, and determine the Michaelis constants in each case. In all cases the intermediate compound of enzyme and substrate consisted of equimolecular proportions of enzymes and protein. The heat inactivation of asclepain *m* and solanain followed the course of a first-order reaction and these enzymes closely resembled papain and bromelin in their high critical thermal increments.

Growth Substances.

Higher Plants.—The mechanism of the pea test for auxin has been further investigated by F. W. Went,⁴⁸ who finds that the curvature of split etiolated pea stems under the action of the auxin is due to a loss of sensitivity of the tissues bordering the wound. This loss of sensitivity is essential before auxin effect will take place, and is of the nature of a preparatory action, which is independent of p_{H} and may be effected by substances which lack growth-promoting activity. The dual aspect of auxin action is again referred to by F. W. Went,⁴⁹ who distinguishes two phases in the action of indolyl-acetic acid on etiolated pea stem cuttings. There is an initial effect which causes a redistribution of rhizocaline, and such effect is brought about by phenylacetic acid, which is not in itself a growth promoter; the later effect is induced only by indolylacetic acid and its homologues and appears to involve the activation of accumulated rhizocaline.

K. V. Thimann and C. L. Schneider⁵⁰ have shown that the relative activities of growth substances as compared with indolyl-acetic acid vary with the species of plant treated and with different methods of treatment in the same species. They record results with a number of growth substances, and employ straight growth tests

⁴⁶ *J. Biol. Chem.*, 1940, **135**, 775.

⁴⁷ *Ibid.*, p. 781.

⁴⁸ *Bull. Torrey Bot. Club*, 1939, **66**, 361.

⁴⁹ *Amer. J. Bot.*, 1939, **26**, 24.

⁵⁰ *Ibid.*, pp. 328, 792.

with *Pisum*, and a new method of curvature test wherein *Avena* coleoptiles are slit longitudinally and thus grown in auxin solutions. It is claimed that the test is some 30 times as sensitive as the agar block method, and that indolylacetic acid may be detected in concentration as low as 0·01 µg. per litre.

That auxin is present in bound form which is only slowly split up by an action which is probably enzymic is suggested by F. Skoog and K. V. Thimann.⁵¹ They find that extraction of auxin with ether is complete only after some months and it is assumed that this is due to the slow hydrolysis of auxin in the bound form. That the mechanism may be enzymic is indicated by the fact that addition of trypsin preparations accelerates the extraction according to the particular preparation employed.

D. M. Bonner⁵² has shown that acids of similar molecular structure have the same activity in growth reactions, dependent on p_H . Thus, the acid-induced curvature in split sections of pea stems results from an increase in active auxin produced after a change in the internal p_H of the cut surface. Dissociation measurements on a number of acids such as *cis*-cinnamic, indolyl-acetic, -propionic, and -butyric, and naphthylacetic show that equimolar concentrations of the acids have the same activities. The effect of p_H in respect of the growth reaction of *Avena* coleoptiles has been examined by J. V. Rakitin and L. M. Jarkovaja,⁵³ who find that increasing acidity over the range 6·32—3·8 enhances the auxin effect. Observations were made with an acetate buffer and with oxalic, citric, malic, and sulphuric acids.

N. H. Grace⁵⁴ has compared the activity in inducing rooting of cuttings shown by acids of the ω -naphthyl-aliphatic series, and finds activity for acids up to and including the hexoic acid. Acids having an even number of carbon atoms in the side chain have a greater activity than those with an odd number. In experiments on rooting of cuttings of *Lonicera tartarica*, indolylbutyric acid was most effective; indolylacetic and indolylpropionic acids showed less activity, and 5-methylindolylpropionic and indolylvaleric acids no action at all.

A. E. Hitchcock and P. W. Zimmerman⁵⁵ have examined the combined effect of mixtures of root-inducing and other substances and find that in a number of cases the mixtures are more effective than the separate components. Such mixtures frequently give greater numbers of roots, a higher percentage of rooted cuttings and

⁵¹ *Science*, 1940, **92**, 64.

⁵² *Bot. Gaz.*, 1938, **100**, 200.

⁵³ *Compt. rend. Acad. Sci., U.R.S.S.*, 1939, **23**, 952.

⁵⁴ *Canadian J. Res.*, 1939, **17**, C, 247, 373.

⁵⁵ *Contr. Boyce Thompson Inst.*, 1940, **11**, 143.

concomitant phenomena associated with large amounts of growth substances. Mixtures of indolyl-acetic and -butyric acids, and of naphthyl- and phenyl-acetic acids with vitamins B₁ and B₆ and ethylene were used. It appears that the vitamins have no root-inducing function in themselves, but act rather as root formation activators.

It has been recognised for a long time that the concentration of heteroauxin applied to the plant is somewhat critical, and that in general higher concentrations not merely have little effect, but may actually inhibit those effects which in lower concentrations are promoted. This finds support in experiments by Y. Hwang and H. L. Pearse,⁵⁶ and by L. Duhamet.⁵⁷ The former found that dilute indolylacetic acid had little effect on oat and bean seedlings, and that concentrations higher than 2 parts per million retarded growth. Indolylacetic acid is evidently only a growth stimulator when natural auxin is deficient. The latter worker, employing extremely low concentrations of indolylacetic acid, finds no effect with the lowest, double the growth rate with a slightly less dilute solution, and inhibition above a certain concentration. Growth of roots of *Lupinus albus* was observed in these experiments, and the small amounts necessary to induce the auxin effect may be gauged when it is stated that the best response was obtained with solutions of 10⁻¹¹ normality.

Growth substances applied in vapour form were found to give the same characteristic responses as when applied as aqueous solutions, by P. W. Zimmermann, A. E. Hitchcock, and F. Wilcoxon.⁵⁸ Similar experiments with vapours and solutions of growth substances were later described by P. W. Zimmermann and A. E. Hitchcock,⁵⁹ who examined 54 substances, including 26 reported for the first time. All produced formative effects, although these differed in character with the substance used. It was notable that the more active substances employed in vapour form produced emanations from the treated plants which affected their neighbours. After a period of one hour from exposure to the vapour, when carbon dioxide production fell below that of controls, the production for the next five hours exceeded that of the controls. The same authors also compared the efficiency of application of root-inducing substances as dusting powders and in solution. The two methods appear to have about the same efficiency, but it was found that in the case of application in a talc dusting powder, the talc itself had some beneficial influence, in part due to the improvement in water relationships and in part

⁵⁶ *Ann. Bot.*, 1940, **4**, 31.

⁵⁷ *Compt. rend.*, 1939, **208**, 1838.

⁵⁸ *Contr. Boyce Thompson Inst.*, 1939, **10**, 363.

⁵⁹ *Ibid.*, pp. 481, 461.

possibly due to the fact that talc appears to contain a physiologically active ingredient, which could be extracted by chloroform.

Further investigation⁶⁰ of the traumatin (wound hormone) isolated by J. English, J. Bonner, and A. J. Haagen-Smit⁶¹ has shown it to be Δ^1 -decene-1 : 10-dicarboxylic acid, and the name traumatic acid has been given. The acid promotes wound periderm formation in potato and inhibits germination in seeds of tomato. In this respect decanedicarboxylic acid and sebacic acid were found to be about half as active as traumatic acid.

A new growth substance is reported by S. C. Bausor,⁶² who has found that naphthoxyacetic acid and its sodium and potassium salts induce typical responses when applied in lanoline paste. The effects were recorded in detail in the case of root primordia of tomato plants.

A plant growth inhibitor was discovered by W. S. Stewart, W. Bergren, and C. E. Redemann⁶³ in cotyledons of radish plants, and a similar substance is reported by R. H. Goodwin⁶⁴ in ethereal extracts of maize meal and bean shoots; further confirmation of the existence of substances which inhibit growth, or at least mask the action of auxin, is provided by R. Snow⁶⁵ by the isolation of a water-soluble substance from pea leaves soaked in ether.

Yeast.—The final elucidation of structure and synthesis of pantothenic acid has been reported.⁶⁶ The high structural specificity of pantothenic acid is indicated as the result of biological examination of amino-acid analogues of the acid carried out by H. H. Weinstock, E. L. May, A. Arnold, and D. Price.⁶⁷ Esters of aspartic acid, alanine, lysine, and β -aminobutyric acid were prepared and condensed with $\alpha\gamma$ -dihydroxy- $\beta\beta$ -dimethylbutyric acid—the non-nitrogenous moiety of pantothenic acid. None of the synthetic analogues was active, although the close relationship of the amino-acids mentioned to β -alanine is sufficiently obvious.

E. E. Snell, R. E. Eakin, and R. J. Williams⁶⁸ have utilised the growth response of *Sacch. cerevisiae* in presence of β -alanine and vitamin B₆ to determine minute amounts of biotin in natural materials such as autolysed liver, whey solids and cane molasses, which are among the richest sources of biotin. The latter is thought to be an α -amino-acid. A similar biological method of assay applied to pantothenic acid is described by D. Pennington, E. E. Snell, and R. J. Williams,⁶⁹ who utilise the response of *Lactobacillus casei* ε.

⁶⁰ *Science*, 1939, **90**, 329; *J. Amer. Chem. Soc.*, 1939, **61**, 3434.

⁶¹ *Ann. Reports*, 1939, **36**, 369.

⁶² *Amer. J. Bot.*, 1939, **26**, 415, 733.

⁶³ *Science*, 1939, **89**, 185.

⁶⁴ *Amer. J. Bot.*, 1939, **26**, 130.

⁶⁵ *Nature*, 1939, **144**, 906.

⁶⁶ See this vol., p. 226.

⁶⁷ *J. Biol. Chem.*, 1940, **135**, 343.

⁶⁸ *J. Amer. Chem. Soc.*, 1940, **62**, 175.

⁶⁹ *J. Biol. Chem.*, 1940, **135**, 213.

The requirements of yeast for the recognised bios substances are known to vary with the strain of yeast. Further confirmation of this is forthcoming as the result of recent work by R. J. Williams, R. E. Eakin, and E. E. Snell,⁷⁰ who also note that the necessity for these substances appears to diminish after long incubation. They also report the presence in liver extracts of additional unknown stimulants, in support of an earlier paper by E. F. Pratt and R. J. Williams⁷¹ in which it was stated that the respiration and growth of yeast are stimulated to a much greater extent by liver extract than by any other known stimulant. Pantothenic acid not only exerts a stimulating effect on growth and respiration of living yeast but has been shown to enhance fermentation by dialysed yeast maceration juice. Suggestions that liver extract contains a yeast stimulant find support from the work of B. Alexander and Y. Subbarow,⁷² who have obtained an acetone extract from liver which is active as a bios substance but appears on biological test to contain a substance different from any of those at present recognised as yeast growth stimulants. It is stable to heat and to acid hydrolysis and to some extent to alkaline hydrolysis, and is soluble in organic solvents. It is precipitated by phosphotungstic acid and unaffected by nitrous acid at room temperature.

F. W. N.

9. INDUCED GLYCOSIDE FORMATION IN PLANTS.

The full significance of glycosides in higher plants is still far from being understood. It has long been considered that the glycosides represent a detoxication mechanism in the plant analogous to the glucuronides excreted by animals when foreign substances are absorbed. The natural aglycones in plants may be said, on this view, to be toxic by-products of metabolism held in an immobilised state because the plant has no excretory system. It has now been shown by L. P. Miller¹ that a variety of plants will form glycosides from certain foreign substances which had previously² been shown to be physiologically active. This gives experimental support for the view that in the plant glycoside formation is a detoxication mechanism, as is the formation of glucuronides in animals. At present the results cannot be considered directly in relation to the natural glycosides, but the experimental methods used have over-

⁷⁰ *J. Amer. Chem. Soc.*, 1940, **62**, 1204.

⁷¹ *J. Gen. Physiol.*, 1939, **22**, 637. ⁷² *J. Biol. Chem.*, 1940, **135**, 341.

¹ *Contr. Boyce Thompson Inst.*, 1938, **9**, 425; 1940, **11**, 271; *Science*, 1940, **92**, 42.

² F. E. Denny, *Amer. J. Bot.*, 1926, **13**, 118; *Contr. Boyce Thompson Inst.*, 1937, **8**, 473.

come former difficulties and open a wide field for further investigation. Ethylene chlorohydrin, one of the substances found to break the dormancy of tubers, is converted by the living tissue into β -2-chloroethyl-*d*-glucoside. The same plant was shown to convert *o*-chlorophenol into β -(*o*-chlorophenyl)gentiobioside. Chloral hydrate was converted into β -trichloroethylgentiobioside, reduction to the alcohol taking place as in animals. Thus it appears that the foreign substance influences the type of glycoside subsequently obtained from the plant. That the foreign aglycones are literally immobile in the plant is shown by the fact that on the subsequent growth of treated corms no traces of the induced glycoside can be found either in the tops or in the new corm.

R. H.

10. GROWTH FACTORS FOR BACTERIA.

Accessory food factors playing a part in the nutrition of the animal¹ and of yeasts² have recently been dealt with in these Reports; the subject of bacterial vitamins will now be reviewed.

Most of the vitamins known to function in the animal and all those so far found necessary for any variety of yeast have been found necessary for one bacterium or another; on the other hand, several substances have been shown to be necessary constituents in the growth of bacteria but have not yet been shown to function in the animal. It may be recalled, however, that pantothenic acid was proved to be a necessary factor in the nutrition of certain yeasts before it was proved to function in the animal.

It is well known that heterotrophic bacteria differ widely in their food requirements. Many organisms isolated from soil can be cultivated in serial subculture on media consisting of inorganic salts, deriving their nitrogen requirements from ammonia and their carbon from carbohydrates or salts of simple organic acids. Others need complex media consisting of protein digests with or without carbohydrate; for still more exacting organisms, blood, ascitic fluid, liver extract, yeast autolysate or egg yolk may be necessary; it is mainly the last type which provides the material for vitamin studies. According to the view put forward by P. Fildes,³ organisms which require a large selection of compounds from which to build up their cell material have lost a number of synthetic powers which more primitive organisms possess; this he believes is due to prolonged cultivation in an environment where they are surrounded by a rich assortment of molecules which they are able to use "ready made," as a result of which they lose synthetic powers. Thus highly para-

¹ *Ann. Reports*, 1939, **36**, 340.

² *Ibid.*, p. 369.

³ *Proc. Roy. Soc. Med.*, 1934, **28**, 79.

sitic organisms resident in the animal body have more exacting food requirements when isolated and grown in culture than have most of their near relatives living in soil. Even non-parasitic organisms such as the lactic fermenters have in many cases lost the power to synthesise essential molecules, such as riboflavin or pantothenic acid, which are present in milk.

The inability to synthesise substances essential for the building of cell material is carried to greater lengths among bacteria than in the animal world. Not only is the list of accessory food factors already known for bacteria longer than that for the animal, but many strains must be supplied with a formidable selection of amino-acids, some in only very low concentration. For such organisms these amino-acids cannot logically be distinguished in function from vitamins, though for convenience it is proposed to exclude them from this Report.

In investigating the nutritional requirements of an exacting organism, two methods of approach are possible. A medium may be built up from simple known constituents until it is adequate for the growth of the organism. This method is possible only if the requirements of the organism are comparatively simple. The second method, and the one more likely to attain success, is to start with a medium—however complicated—fully adequate for the growth through serial subculture, and then, by fractionating the various constituents, eliminate unnecessary material until the simplest group adequate has been attained. If this still contains unknown constituents, it may be possible to replace them by known vitamins; otherwise the active substances must be isolated and their constitution determined. When this has been achieved, two concentrations of the new factor should be given; the minimum concentration which supports visible growth and the minimum required for maximum growth through serial subculture.

The first method is exemplified by work of Fildes on *Proteus*, the second by the work of Mueller on *C. diphtheriae*.

Proteus.—This organism differs from those with the simplest requirements in only one particular, that is, it grows aerobically on inorganic salts and ammonium lactate if nicotinic acid only is added ($0.1 \mu\text{g./ml.}$).⁴ Nicotinic acid is among the most widespread requirements of bacteria and it is possible that the ability to synthesise it is readily lost. Within the *Proteus* group, however, two degrees of synthetic disability are found, all *P. morganii* strains requiring in addition pantothenic acid, which produces detectable growth in $0.001 \mu\text{g./ml.}$ and optimal growth in $0.005 \mu\text{g./ml.}$ ⁵

⁴ P. Fildes, *Brit. J. Exp. Path.*, 1939, **19**, 239.

⁵ M. J. Pelczar and J. R. Porter, *Proc. Soc. Exp. Biol. Med.*, 1940, **43**, 151.

The second or analytical method for determining the growth requirements of a microbe is exemplified in the classical studies of J. H. Mueller⁶ on the exacting, H.Y. strain of *C. diphtheriae*. This organism was cultivated on a medium consisting of (A) a salt mixture, (B) Liebig's extract, 7.5 mg./ml.; (C) tryptophan, 0.1 mg./ml.; (D) an acid hydrolysate of caseinogen, 10 mg./ml. The growth obtained in 60 hours was equivalent to 0.2 mg. of nitrogen/ml. and it was the aim of the study to replace this medium by one of known composition giving an equal bacterial crop in the same time. (A), (B), and (C) being kept constant, (D) was adequately replaced by a mixture of amino-acids with ethyl alcohol as additional source of energy; the acid hydrolysate of casein being now replaced by known compounds free from contaminating vitamins, (B) was replaced by liver extract, from which were isolated two constituents both necessary for the growth of the organism, *viz.*, nicotinic acid,⁷ active in 0.1 µg./ml., and β-alanine,⁸ active in 0.1 µg./ml.; a third substance necessary was present in the liver extract, but was actually isolated from cow's urine; this was pimelic acid, active in 0.005 µg./ml.⁹ The effect of the last is duplicated by synthetic pimelic acid, but not by any other member of the series tried. These three substances in the concentrations stated completely replaced the liver extract. These findings were confirmed by English workers¹⁰ for all strains of *C. diphtheriae mitis* and most of the *gravis* strains tested. Some still more exacting strains of the latter type failed to grow on this medium, but grew when liver extract was added. From the latter a concentrate was prepared which could be replaced by pantothenic acid¹¹ ($\alpha\gamma$ -dihydroxy- $\beta\beta$ -dimethylbutyrylalanide). We have then in this group two grades of synthetic disability; the majority of strains can use either β-alanine or pantothenic acid, the exacting *gravis* strains require pantothenic acid supplied as such.

The Lactic Fermenters.—This group of organisms, isolated from milk and cheese, forms another group of varying vitamin requirements. Orla-Jensen *et al.*¹² first showed that milk which had been shaken with activated charcoal no longer supported the growth of many of these strains, but that when riboflavin together with the

⁶ *J. Bact.*, 1935, **29**, 515.

⁷ J. H. Mueller, *J. Biol. Chem.*, 1937, **120**, 219.

⁸ J. H. Mueller and S. Cohen, *J. Bact.*, 1937, **34**, 381.

⁹ J. H. Mueller, *J. Biol. Chem.*, 1937, **119**, 121.

¹⁰ W. C. Evans, W. R. C. Handley, and F. C. Happold, *Brit. J. Exp. Path.*, 1939, **20**, 41, 396.

¹¹ E. T. Stiller, J. C. Keresztesy, and J. Finkelstein, *J. Amer. Chem. Soc.*, 1940, **62**, 1779.

¹² S. Orla-Jensen, N. C. Otte, and A. Snog-Kjaer, *Mem. Acad. Roy. Sci. Lettres Danemark*, 1936, **6**, no. 5; *Zentr. Bakt. Par.*, II, **94**, 434, 452.

material eluted from the charcoal was added to the deficient milk, the growth rate attained on untreated milk was almost reached. Later workers have more precisely determined the requirements of this group;¹³ the medium consisted of bactopeptone, salts, cystine, and glucose. This was rendered free from riboflavin by exposing the peptone to light in alkaline solution; four strains grew on the riboflavin-free medium (*Str. lactis*, *L. arabinosus*, *L. pentosus*, and *Leuconostoc mesenteroides*); *L. delbrückii*, *L. casei*, *L. gayonii*, and *B. lactis acidi* grew only when riboflavin (0.1 µg./ml.) was added. It is noteworthy that all lactic fermenters tested contain riboflavin whether it is supplied in their growth medium or not.¹⁴

The activity of various synthetic flavins was tested and compared with that of the natural product obtained from milk; only those containing ribose were active and none equalled riboflavin.¹⁵

	<i>Activity.</i>
I. 6 : 7-Dimethyl-9-d-l'-ribitylisoalloxazine (riboflavin) ...	100
II. 6-Methyl-9-d-l'-ribitylisoalloxazine	50
III. 7-Methyl-9-d-l'-ribitylisoalloxazine	78
IV. 7-Methyl-6-ethyl-9-d-l'-ribitylisoalloxazine	75
V. 6 : 7-Dimethyl-9-d-l'-arabitylisoalloxazine	Inactive
VI. 6 : 7-Dimethyl-9-l-l'-arabitylisoalloxazine	"
VII. 6 : 7-Dimethyl-9-1'-sorbitylisoalloxazine.....	"
VIII. 9-ll-Arabitylisoalloxazine	"
IX. 9-(dl-Ribityl)-5 : 6-benzoisoalloxazine	"
X. 6 : 7 : 9-Trimethylisoalloxazine (lumiflavin)	"
XI. 6 : 7-Dimethylalloxazine (lumichrome)	"
XII. Riboflavin tetra-acetate	"

It is noteworthy that substitution in the 6 : 7-positions leaves some activity and that the ribityl group is essential and cannot be replaced by other sugars or by methyl.

The effectiveness of the synthetic flavins for bacterial growth is closely paralleled in animal studies, only I, II, III, and IV being active in rat growth tests.¹⁶

The necessity for pantothenic and nicotinic acids for some members of the group was demonstrated as follows. A medium consisting of an acid hydrolysate of bactopeptone supplemented by salts, sodium acetate, cystine, and riboflavin failed to support growth unless liver extract was added. The latter was fractionated, and a concentrate obtained active in 0.003 µg./ml. This was subsequently found to owe its activity to pantothenic acid. Some strains grew poorly on this medium, and required in addition nicotinic acid (0.05 µg./ml.). Strains requiring pantothenic acid were *B. lactis*

¹³ E. E. Snell, F. M. Strong, and W. H. Peterson, *Biochem. J.*, 1937, **31**, 1789; *J. Bact.*, 1939, **38**, 293; *J. Amer. Chem. Soc.*, 1938, **60**, 2825.

¹⁴ F. Schütz and H. Theorell, *Biochem. Z.*, 1939, **295**, 246.

¹⁵ E. E. Snell and F. M. Strong, *Enzymologia*, 1939, **6**, 186.

¹⁶ Kühn *et al.*, *Ber.*, 1937, **70**, 2560.

acidi, *L. arabinosus*, *L. pentosus*, *L. delbrückii*, *B. brassicæ* and *Str. lactis*. Those requiring nicotinic acid in addition were *L. casei* and *L. arabinosus*.¹⁷

The strain of *Str. lactis* isolated from silage and originally known as *Bact. acetyl choline* (Keil), in addition to factors already mentioned, requires adermin (vitamin B₆), 3-hydroxy-4 : 5-bis(hydroxymethyl)-2-methylpyridine.¹⁸ This compound is also required by *Str. haemolyticus* (see p. 440).

The Dysentery Group.—S. A. Koser *et al.*¹⁹ have shown that nicotinic acid is essential for a number of dysentery bacilli (*Flexner*, *Hiss*, *Strong*, and other unspecified strains). These were sown in a basal medium of fifteen amino-acids, glucose, and salts; growth was completely negative on the basal medium, but full rapid growth occurred on the addition of nicotinic acid, 0·1 µg./ml.; 0·04 µg./ml. gave slower growth and a visible effect was obtained with 0·01 µg./ml.

The Pasteurella Group.—A number of organisms of the *Pasteurella* group have been found to require nicotinamide and pantothenic acid (0·1 µg./ml. was used in both cases); the former was replaceable by coenzyme I, but β-alanine did not replace pantothenic acid; this case resembles that of the exacting strains of *C. diphtheriae gravis*.²⁰

The Staphylococci.—The growth requirements of this group of organisms are high. Aerobically they grow on peptone water, but it is clear that this does not function only as a source of amino-acids, for when it is replaced by (say) an acid digest of caseinogen plus tryptophan, tyrosin, and cystine, no growth occurs, unless extract of meat or yeast is added. From the latter (in the form of marmite) two active fractions, both necessary, were obtained,²¹ the one replaceable by nicotinic acid or amide (0·2 µg./ml.) or by diphosphopyridinedinucleotide, the other by aneurin (0·02 µg./ml.).

The organism can use the two basic components of aneurin if these are provided separately, *viz.*, 4-amino-5-aminomethyl-2-methylpyrimidine (0·002 µg./ml.) and 4-methyl-5-β-hydroxyethylthiazole (about 0·01 µg./ml.).

The specificity for the thiazole base appears to be complete, closely related compounds tried being quite inactive even at concentrations 100 to 1000 times that used for the acceptable compound. Thiochrome, for example, will not replace aneurin and 4-methyl-

¹⁷ E. E. Snell, F. M. Strong, and W. H. Peterson, *J. Amer. Chem. Soc.*, 1938, **60**, 2825.

¹⁸ E. F. Möller, *Z. physiol. Chem.*, 1938, **254**, 285.

¹⁹ S. A. Koser, A. Dorfman, and F. Saunders, *Proc. Soc. Exp. Biol. Med.*, 1938, **38**, 311.

²⁰ S. Berkman, F. Saunders, and F. A. Koser, *ibid.*, 1940, **44**, 68.

²¹ B. C. J. G. Knight, *Biochem. J.*, 1937, **31**, 731.

thiazole does not replace 4-methyl-5- β -hydroxyethylthiazole. The organism is, however, less specific towards the pyrimidine group, the following substitutes for the aneurin base being active in approximately the same concentration, *viz.*, 4-amino-5-thioformamido-methyl-2-methylpyrimidine and 4-amino-5-aminomethyl-2-methyl-pyrimidine; inactive, however, were 4-hydroxy-5-hydroxymethyl-2-methylpyrimidine, 4-hydroxy-5-aminomethyl-2-methylpyrimidine, and 4-amino-2-hydroxypyrimidine (cytosine).²²

The anaerobic metabolism of *Staph. aureus* is different from its aerobic metabolism; in the former case it derives its energy mainly from amino-acids; anaerobically from glucose and pyruvic acid. In the latter case an additional factor is required, *viz.*, uracil (2 : 6-dihydroxypyrimidine), which is active in 1—5 $\mu\text{g}./\text{ml}$. Related bases were inactive, *viz.*, 5-methyluracil (thymine), 4-methyluracil, 1 : 3-dimethyluracil, 1 : 3 : 4-trimethyluracil, 2-thio-5-methyluracil, barbituric acid, cytosine, and *isocytosine*.²³

Str. haemolyticus.—Growth of this organism failed on a medium consisting of bactopeptone to which were added cystine, glucose, and a formidable list of bacterial vitamins. The addition of meat extract supplied the material lacking, which was subsequently identified as glutamine, which supported full growth at 30 $\mu\text{g}./\text{ml}$.²⁴ A number of related compounds, including glutamic acid, aspartic acid, and asparagine, are inactive.²⁵

Two other factors for this organism were discovered by treating the bactopeptone with alkali, which resulted in an inactive medium. Growth was then obtained on the addition of riboflavin (0.1 $\mu\text{g}./\text{ml}$) and pantothenic acid (1.0 $\mu\text{g}./\text{ml}$).^{26, 27} Finally, vitamin B₆ was shown to be necessary by growing the organism on a selection of amino-acids (replacing protein hydrolysate), riboflavin, and pantothenic acid plus an aqueous extract of liver. The active part of the last was adsorbed on lead sulphide and eluted, and the eluate adsorbed on and eluted from fuller's earth. The concentrate thus obtained was active in 1 $\mu\text{g}./\text{ml}$. and could be replaced by vitamin B₆.²⁸ This was confirmed by an alternative procedure.²⁹

The Clostridia.—The requirements of the *Clostridia* (spore-bearing strict anaerobes) are notoriously high as to both vitamins and amino-

²² B. C. J. G. Knight, *Biochem. J.*, 1937, **37**, 966.

²³ G. M. Richardson, *ibid.*, 1936, **30**, 2184.

²⁴ H. McIlwain, P. Fildes, G. P. Gladstone, and B. C. J. G. Knight, *ibid.*, 1939, **33**, 223.

²⁵ H. McIlwain, *ibid.*, p. 1942.

²⁶ B. L. Hutchings and D. W. Woolley, *J. Bact.*, 1938, **38**, 285.

²⁷ H. McIlwain, *Brit. J. Exp. Path.*, 1939, **20**, 330.

²⁸ B. L. Hutchings and D. W. Woolley, *Science*, 1939, **90**, 42.

²⁹ H. McIlwain, *Brit. J. Exp. Path.*, 1940, **21**, 25.

acids and few cases have been worked out in detail. *Cl. sporogenes* furnished an early example of a bacterial vitamin.³⁰ The active substance was found in yeast, moulds, bacteria, and urine; a highly concentrated preparation from yeast was active in 0.02 µg./ml. It is an acid substance forming a soluble barium salt and a methyl ester distilling at 80—100°/0.001 mm.³¹ It awaits further identification and is probably necessary for other members of the *Clostridia*.

The butyl fermenters display definite vitamin requirements and work on this group is in a state of active progress. *Cl. butylicum* grows on a synthetic medium containing asparagine and glucose as the only organic compounds and needs in addition only biotin.^{32, 33, 34} It can be used, therefore, as the test organism for the presence of this substance, 1.3×10^{-5} µg./ml. being detectable. The following table shows growth as measured by the turbidimeter; 0 represents the uninoculated culture, 100 complete opacity.

Biotin, µg./ml.	Growth measured by turbidimeter.
0.000000	2.2
0.0000133	10.2
0.0000266	29.0
0.000053	54.0
0.00010	75.0
0.00020	88.0
0.00066	94.0
 Liver conc., µg./ml.	
0.0133	39.0
0.0333	60.0
0.0666	79.0
0.1332	94.0
0.2664	94.0
0.6660	96.0

The closely related *Cl. acetobutylicum* requires in addition some factor or factors obtained from yeast.^{35, 36} Moreover, the study of these fermenters is complicated by the fact that some still unidentified factor (or factors) modifies the course of the fermentation as well as the growth.^{36, 37}

The Hæmophilus Group.—The vitamin requirements of this group have not been determined recently enough to be considered here and will therefore be only briefly stated. Two factors were found necessary in cultivating these organisms, the X factor obtained from

³⁰ B. C. J. G. Knight and P. Fildes, *Brit. J. Exp. Path.*, 1933, **14**, 112.

³¹ A. M. Pappenheimer, *Biochem. J.*, 1935, **29**, 2055.

³² F. Kögl and B. Tönnis, *Z. physiol. Chem.*, 1936, **242**, 43.

³³ E. E. Snell and R. J. Williams, *J. Amer. Chem. Soc.*, 1939, **61**, 3594.

³⁴ W. H. Peterson, L. E. McDaniel, and E. McCoy, *J. Biol. Chem.*, 1940, **133**, LXXXV.

³⁵ C. Weizmann and B. Rosenfeld, *Biochem. J.*, 1939, **33**, 1376.

³⁶ A. E. Oxford, J. O. Lampen, and W. H. Peterson, *ibid.*, 1940, **34**, 1588.

³⁷ C. Weizmann and B. Rosenfeld, *loc. cit.*

blood and the V factor from animal or plant tissues or other bacteria. The former was identified as haematin, and the latter as diphosphopyridinenucleotide (coenzyme I), replaceable by the triphospho-derivative (coenzyme II). When grown anaerobically, the former can be dispensed with and it has been suggested that it is required, in part at any rate, for the synthesis of catalase, which is unnecessary in anaerobic life where hydrogen peroxide is not formed. It has recently been claimed that cysteine can replace haematin in aerobic growth; this is regarded as evidence that the haematin is required for the synthesis of catalase; in the presence of cysteine hydrogen peroxide would be reduced and catalase rendered unnecessary. Further details of this work are promised.³⁸

Nicotinic acid or amide cannot replace the diphosphopyridine-nucleotide in this group, whereas organisms requiring the former can replace it by the latter (cf. the case of β -alanine and pantothenic acid in the *diphtheriae* group).

Functions of Bacterial Vitamins.—Organisms which require a given vitamin can be used as delicate reagents for the detection of that substance in naturally occurring materials and for a rough quantitative assay during its isolation. *Str. haemolyticus* or one of the exacting strains of *C. diphtheriae gravis* can be used to determine the presence and approximate amount of pantothenic acid in a liver or yeast extract, the only alternative method involving prolonged animal feeding experiments; six days' work may thus replace six weeks'. It is fairly apparent that the vitamins shown to be necessary in the special strains which require them supplied in the medium are of wide-spread importance and exist also in other species which are able to make them for themselves. This can be shown by using extracts of non-exacting strains to supply the known requirements of exacting strains.

Some success has been achieved in determining the function of certain vitamins by the method first used by Lwoff and Lwoff. This consists in growing an organism requiring the vitamin in a medium containing it in a sub-optimal concentration. The organism so obtained is comparable with a vitamin-deficient animal and by a comparison of its enzyme systems with those of the normally grown organism it may be possible to show what chemical mechanism is deficient. Thus *H. parainfluenzae* grown in sub-optimal amounts of coenzyme I was shown to have decreased powers of oxidising glucose, etc.; this power could be augmented by the addition of coenzyme I to the reaction vessel. With the organism grown normally, the addition of coenzyme I does not affect the oxidation rate.³⁹

³⁸ T. L. Snyder and R. H. Broh-Kahn, *Nature*, 1938, **143**, 153.

³⁹ A. Lwoff and M. Lwoff, *Proc. Roy. Soc.*, 1936, **B**, **122**, 360.

Washed suspensions of *Staph. aureus* grown in sub-optimal amounts of aneurin oxidise and dismutes pyruvate at a lower rate than suspensions of organisms grown in optimal amounts of aneurin; the rates in the former case are increased by the addition of aneurin to the reacting vessel, but the rates in the latter case are not affected.⁴⁰ The organisms grown in the aneurin-deficient medium are seen, therefore, to suffer from lack of cocarboxylase (aneurin diphosphate) and to display the same decreased ability to metabolise pyruvate as the tissues of the pigeon suffering from the same vitamin deficiency.

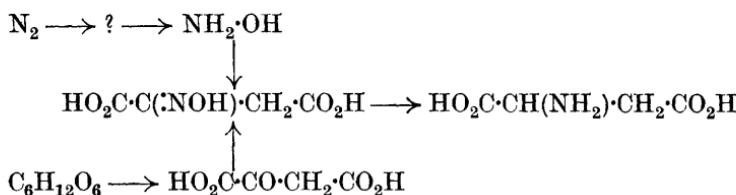
The following table indicates the vitamin requirements among bacteria, yeasts, and animal tissues :

	Bacteria.	Yeasts.	Animal.
Nicotinic acid.....	+	+	+
β -Alanine	+	+	
Pantothenic acid	+	+	+
Riboflavin	+	+	+
Adermin (vitamin B ₆)	+	+	+
Aneurin	+		+
Uracil	+		
Asparagine	+		
Biotin	+	+	+
Sporogenes vitamin	+		
Coenzyme I	+		
Hæmatin	+		
Pimelic acid	+		

M. S.

11. NITROGEN METABOLISM OF BACTERIA.

Nitrogen Fixation.—The publication by A. I. Virtanen and T. Laine¹ of the experimental details of their work on the symbiotic nitrogen-fixing *Rhizobium* confirms the general scheme set out in the former's book² and collects the information appearing in various notes and abstracts.³ It is claimed that nitrogen is fixed by the organism with the production of hydroxylamine, which then reacts with oxaloacetic acid produced by the host plant from carbohydrate, as follows :



⁴⁰ G. M. Hills, *Biochem. J.*, 1938, **32**, 383. ¹ *Biochem. J.*, 1938, **32**, 412.

² A. I. Virtanen (1938), "Cattle Fodder and Human Nutrition," Cambridge University Press.

³ A. I. Virtanen, *J. Soc. Chem. Ind.*, 1935, **54**, 1015; *J. Agric. Sci.*, 1937, **27**, 332; *Agric. Col. Sweden Ann.*, 1938, **5**, 429.

The evidence supporting this scheme, which is put forward by these workers, rests on the following points :

(1) The infected plant excretes *l*-aspartic acid into the medium around the roots.^{4, 5}

(2) The aspartic acid is excreted only from roots infected with *Rhizobium*.⁶

(3) Oxaloacetic acid can be detected in the host plant.⁷

(4) The oximinosuccinic acid has been isolated and identified,^{1, 8} but hydroxylamine itself has not been identified.

(5) Nitrogen fixation by free-living *Rhizobium* cultures has been observed in the presence of oxaloacetic acid.⁹

(6) The greater part of the nitrogen excretion which is not accounted for by the aspartic acid consists of β -alanine,¹⁰ and suspensions of *Rhizobium* will decarboxylate aspartic acid to form β -alanine.¹¹

(7) In the presence of crushed pea plants, the amino-group of aspartic acid may be transferred to keto-acids such as pyruvic acid with the formation of α -alanine, etc.¹²

The conclusions of Virtanen have been criticised by P. W. Wilson,¹³ who has reviewed the work of the Wisconsin school in this field. His criticisms, published before the detailed description of Virtanen's work, are not completely justified now and are largely disposed of by the isolation of the oxime.¹ Wilson has been unable to obtain significant nitrogen-fixation by the free-living *Rhizobium* cultures in the presence of oxaloacetic acid. The Wisconsin workers have shown that, the plant and bacteria being treated as one system, the rate of nitrogen fixation is dependent upon the nitrogen pressure,¹⁴ independent of the oxygen pressure over a wide range,¹⁵ and specifically inhibited by hydrogen.¹³

Little advance has been made in our knowledge of nitrogen-fixation by the free-living *Azotobacter*. A. I. Virtanen and T. Laine¹⁶ have found aspartic acid as the chief product of excretion, but as this is much less marked than with *Rhizobium*, the study has not as yet been elaborated. G. Endres¹⁷ has found oximes formed by

⁴ A. I. Virtanen and T. Laine, *Nature*, 1935, **136**, 756.

⁵ *Idem*, *Suomen Kem.*, **B**, 1937, **10**, 32.

⁶ A. I. Virtanen, von Hausen, Synnöve, and T. Laine, *J. Agric. Sci.*, 1937, **27**, 332.

⁷ A. I. Virtanen and T. Laine, *Suomen Kem.*, **B**, 1938, **11**, 25.

⁸ *Idem*, *Nature*, 1938, **142**, 165.

⁹ *Idem*, *Suomen Kem.*, **B**, 1937, **10**, 24.

¹⁰ *Idem*, *ibid.*, p. 2. ¹¹ *Idem*, *Enzymologia*, 1937, **3**, 266.

¹² *Idem*, *Nature*, 1938, **141**, 748. ¹³ *Ergebn. Enzymforsch.*, 1938, **8**, 13.

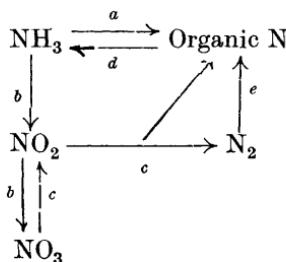
¹⁴ P. W. Wilson, *J. Amer. Chem. Soc.*, 1937, **58**, 1256.

¹⁵ P. W. Wilson and E. B. Fred, *Proc. Nat. Acad. Sci.*, 1937, **23**, 503.

¹⁶ *Suomen Kem.*, **B**, 1937, **10**, 2. ¹⁷ *Annalen*, 1935, **518**, 109; 1938, **535**, 1.

Azotobacter growing in the presence of nitrate. D. D. Woods¹⁸ has shown that washed suspensions of *Cl. welchii* and *Bact. coli* (probably many other types) will reduce nitrate first to nitrite and then to ammonia in the presence of hydrogen and has brought forward good evidence that the reduction of nitrite proceeds through hydroxylamine.

A. S. Corbet and W. R. Wooldridge¹⁹ have investigated the distribution of nitrogenous compounds in sewage and the changes that occur during the activated sludge process. Confirming much earlier work on soil, their experiments indicate that the following interactions and changes can occur in the nitrogen cycle :



The particular course of the reactions followed varies according to the conditions which obtain, especially as regards the proportions of available carbonaceous matter and nitrogen present and the nature of the compound in which the nitrogen occurs. The reactions indicated in the diagram are encouraged by the following chemical conditions :

- (a) The presence of ammonia and assimilable carbon compounds.
- (b) Ammonia present, but little or no assimilable carbon compounds.
- (c) Assimilable carbon compounds present together with nitrite and/or nitrate as the most readily available source of nitrogen.
- (d) Autolytic changes arising from the absence of nutrient materials.
- (e) Fixation of nitrogen in the presence of assimilable nitrogen-free organic compounds and no readily available source of nitrogen other than free gas.

Activated sludges appear to contain the enzymes necessary to effect any of the above changes.

Amino-acid Metabolism.—G. M. Hills²⁰ has studied the amino-acid metabolism of certain pathogenic bacteria. Gram-positive cocci contain an enzyme which attacks arginine to form ornithine and ammonium carbonate ; as neither citrulline nor urea appears to be an intermediate in the reaction, the enzyme has been named arginase

¹⁸ *Biochem. J.*, 1938, **32**, 2000.

¹⁹ *Ibid.*, 1940, **34**, 1015, 1026, 1036.

²⁰ *Ibid.*, p. 1057.

dihydrolase. Gram-positive bacteria produce no ammonia aerobically from other amino-acids with the exception of *Staphylococcus*, which attacks serine and threonine. *Bact. typhosum* deaminates serine, aspartate, threonine, and arginine aerobically, and *C. diphtheriae* appears to attack aspartate only. A. Janke and W. Tayenthal²¹ showed that glycine is oxidatively deaminated by washed suspensions of *Bact. coli*, *Bact. vulgare*, *Ps. fluorescens*, and *Bac. mycoides*: glyoxylic acid was isolated as the 2 : 4-dinitrophenyl-hydrazone. A series of papers by P. Desnuelle *et al.*²² has dealt with the anaerobic breakdown of cysteine and cystine by washed suspensions of *Bact. coli*. Cysteine is broken down by an adaptive cysteinase, liberating ammonia and hydrogen sulphide in equimolecular quantities. The reaction is partially inhibited by glucose and is specific for the natural isomer. Cystine is reduced to cysteine before further attack. The enzyme responsible for the breakdown of cysteine by *Propionibact. pentosaceum*²³ differs from the cysteinase of *Bact. coli* in being accelerated by the presence of glucose, showing no optical specificity, and not requiring the presence of the substrate during growth for its formation. The products of the breakdown other than ammonia and hydrogen sulphide have not been reported. C. E. Clifton²⁴ has shown that serine is disrupted anaerobically by washed suspensions of *Cl. botulinum* with the formation of ammonia, carbon dioxide, acetic acid, and ethyl alcohol. He suggests that pyruvic acid is formed as an intermediate.

The breakdown of *l*(+)-glutamic acid and *l*(-)-aspartic acid by bacteria has been the subject of several investigations. E. Adler and co-workers²⁵ have been able to extract the glutamic acid dehydrogenase from suspensions of *Bact. coli* by a modification of the method used previously by M. Stephenson²⁶ for the extraction of lactic dehydrogenase. The extracted enzyme reduces methylene-blue in the presence of glutamic acid and coenzyme II and evidence is put forward to show that the dehydrogenation to iminoglutaric acid is reversible. The reactions involved in the deamination of glutamic acid are :

- (a) Glutamic acid + coenzyme \rightleftharpoons Iminoglutaric acid + dihydro-coenzyme
- (b) Iminoglutaric acid + H₂O \rightleftharpoons Ketoglutaric acid + NH₃
- (c) Dihydrocoenzyme + $\frac{1}{2}$ O₂ \rightleftharpoons Coenzyme + H₂O

²¹ *Biochem. Z.*, 1937, **289**, 76.

²² P. Desnuelle and C. Fromageot, *Enzymologia*, 1939, **6**, 80, 242, 387.

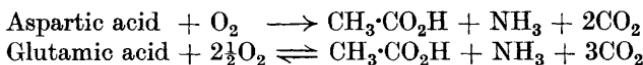
²³ P. Desnuelle, E. Wookey, and C. Fromageot, *ibid.*, 1940, **8**, 225.

²⁴ *Proc. Soc. Exp. Biol. Med.*, 1940, **43**, 588.

²⁵ E. Adler, V. Hellstrom, G. Gunther, and H. Euler, *Z. physiol. Chem.*, 1938, **255**, 14.

²⁶ *Biochem. J.*, 1938, **22**, 605.

J. R. Klein²⁷ has shown that washed suspensions of *Hemophilus parainfluenzae* oxidise aspartic and glutamic acids with the liberation of ammonia and the formation of acetic acid according to the equations :



By a study of the metabolism of possible intermediate compounds, Klein has established the probable course of the oxidation. For aspartic acid the reaction proceeds : aspartic acid → oxalo-acetic acid → pyruvic acid → acetaldehyde → acetic acid. The first and the last step require the presence of a coenzyme.

E. F. Gale²⁸ has shown that there are two mechanisms in *Bact. coli* which deaminate aspartic acid anaerobically. One of the enzymes concerned is stable to toluene treatment and is the aspartase of J. H. Quastel and B. Woolf,²⁹ which deaminates aspartic acid to fumaric acid. The other enzyme is inhibited by toluene and requires the presence of a coenzyme, which can be replaced *in vitro* by adenosine. The two enzymes have been fractionated in a cell-free juice obtained from *Bact. coli* crushed in a bacteria-crushing mill designed by V. H. Booth and D. E. Green.³⁰ Since the fraction containing aspartase II also contains fumarase, the immediate product of the deamination process is not known. A. I. Virtanen and J. Erkama³¹ claim to have shown the presence of two enzymes attacking aspartic acid in *B. fluorescens liquefaciens*, one producing fumaric acid and the other carrying out a hydrolytic deamination to form malic acid. If the latter statement is proved, it will be the first known case of biological hydrolytic deamination.

E. F. Gale and M. Stephenson³² have continued their studies on factors affecting bacterial deamination. Washed suspensions of *Bact. coli* deaminate glycine, *dl*-alanine, and *l*(+)-glutamic acid aerobically and *dl*-serine and *l*(-)-aspartic acid anaerobically. Anaerobic growth conditions increase the formation of the anaerobic deaminases and decrease that of the aerobic deaminases, whereas the presence of glucose during growth inhibits the formation of all deaminases studied to the extent of 85—95%. The activity of the serine deaminase varies greatly with the age of the culture and upon the condition of the organism, the presence of phosphate and a reducing agent being necessary to prevent this activity being lost on standing. J. W. Baker and F. C. Happold³³ have studied the groups essential to the tryptophan molecule for the production of

²⁷ *J. Biol. Chem.*, 1940, **134**, 43.

²⁸ *Biochem. J.*, 1938, **32**, 1583.

²⁹ *Ibid.*, 1926, **20**, 545.

³⁰ *Ibid.*, 1938, **32**, 855.

³¹ *Nature*, 1938, **142**, 954.

³² *Biochem. J.*, 1938, **32**, 392, 1583.

³³ *Ibid.*, 1940, **34**, 657.

indole from that molecule by *Bact. coli*. Up to the present no attempt to isolate or identify an intermediate substance in this breakdown has met with success. By studying the action of the cell-free tryptophanase preparation on a large number of tryptophan derivatives, they have obtained results which suggest that the breakdown to indole requires the following structural features : (a) a free carboxyl group, (b) an unsubstituted α -amino-group, (c) a β -carbon atom capable of oxidative attack. They tentatively suggest a type of breakdown involving "reductive fission" of the tryptophan molecule, but there is as yet no experimental evidence for this type of reaction.

The study of the amino-acid metabolism of the *Clostridia* (strict anaerobes) has been continued by several workers. D. D. Woods and C. E. Clifton^{34,35} have found that *Cl. tetanomorphum* utilises many amino-acids, causing the liberation of ammonia and hydrogen. The course of the breakdown of glutamic acid has been worked out in detail, the products being acetic acid, butyric acid, carbon dioxide, hydrogen, and ammonia. The same products in the same proportions are produced by the growth of an unidentified *Clostridium* growing on a glutamic acid medium and studied by H. A. Barker.³⁶ W. Kocholaty and J. C. Hoogerheide³⁷ have studied the dehydrogenation reactions carried out by *Cl. sporogenes* and have found that in certain cases amino-acid molecules can act as both hydrogen donators and acceptors, so that intramolecular reactions occur in which one molecule is oxidised and another reduced, thus forming a special case of the intermolecular oxidation-reduction reactions discovered by L. H. Stickland.³⁸ C. E. Clifton³⁹ has studied the amino-acid metabolism of *Cl. botulinum* and finds that it obtains its energy through "Stickland" reactions in a manner similar to that of *Cl. sporogenes*.

Amine Formation.—A. I. Virtanen and T. Laine⁴⁰ followed up their observation that *Rhizobium* decarboxylates aspartic acid with the formation of β -alanine by showing that *Bact. coli* produces cadaverine from lysine. Later⁴¹ they found that *Rhizobium* will also decarboxylate glutamic acid to γ -aminobutyric acid. A. H. Eggerth *et al.*⁴² have worked out an improved method for the estimation of histamine in bacterial cultures and A. H. Eggerth⁴³ has investigated the production of histamine by many species of

³⁴ *Biochem. J.*, 1937, **31**, 1774.

³⁵ *Ibid.*, 1938, **32**, 345.

³⁶ *Enzymologia*, 1937, **2**, 175.

³⁷ *Biochem. J.*, 1938, **32**, 437, 949.

³⁸ *Ibid.*, 1934, **28**, 1746.

³⁹ *J. Bact.*, 1940, **39**, 485.

⁴⁰ *Enzymologia*, 1937, **3**, 266.

⁴¹ A. I. Virtanen, P. Rintala, and T. Laine, *Nature*, 1938, **142**, 674.

⁴² A. H. Eggerth, R. S. Littwin, and J. V. Deutsch, *J. Bact.*, 1939, **37**, 187.

⁴³ *Ibid.*, p. 205.

organisms growing in culture. He has shown that many of the common inhabitants of the intestine will produce histamine, especially if glucose is present in the growth medium. By adjusting the medium p_{H} during growth, he showed that histamine is best produced if the p_{H} is low. Growth temperature also plays an important part, as some organisms produce more histamine if grown at a low temperature than at the normal 37°. All these results were obtained by bacteria growing in various media, so the effects studied may be produced by action on the growth of the organism rather than on the histamine-producing mechanism. The conditions under which bacteria produce certain amines by the simple decarboxylation of the corresponding amino-acids have been cleared up in a series of papers by E. F. Gale.⁴⁴ In the first case the conditions under which the organisms are grown are important; to obtain organisms possessing strongly active amino-acid decarboxylating enzymes, they must be grown under acid conditions in the presence of the free amino-acids. The most effective way of doing this is to grow the organisms in a tryptic digest of casein with 2% of glucose, the fermentation of the glucose producing the necessary low p_{H} . Organisms grown in this manner will decarboxylate certain amino-acids quantitatively to the corresponding amines at low p_{H} values, the optimum value in each case depending upon the amino-acid concerned. Thus washed suspensions of *Bact. coli* grown in glucose broth or in broth at p_{H} 5, the physiological limit of growth, will decarboxylate *l*(+)-arginine to agmatine optimally at p_{H} 4·0; *l*(+)-lysine to cadaverine at p_{H} 4·5; *l*(+)-ornithine to putrescine at p_{H} 5·0; *l*(-)-histidine to histamine at p_{H} 4·0; and *l*(+)-glutamic acid to γ -aminobutyric acid at p_{H} 4·0. Similarly, *Streptococcus faecalis* quantitatively decarboxylates *l*(-)-tyrosine to tyramine at p_{H} 5·0. In all cases the product has been isolated from a simple mixture of washed suspension of organism, appropriate buffer, and amino-acid, the quantities so arranged that the decarboxylation has proceeded to completion. Good yields of pure product are obtained and it would seem that this biological method may be the best method for the large-scale production of some of these compounds. Several groups of organisms have been studied from this point of view and again the strictly anaerobic group of *Clostridia* proves interesting: *Cl. welchii* decarboxylates histidine to histamine at the exceptionally low optimum p_{H} of 2·5–3·0, so in this case appreciable amounts of histamine are only produced *in vivo* when the organism grows in the presence of fermentable carbohydrate. Amongst other members of the group, *Cl. septique* decarboxylates ornithine to putrescine at p_{H} 5·5 and *Cl. aerofætidum* forms tyramine from tyrosine at p_{H} 5·0. Many organisms, e.g., most

⁴⁴ *Biochem. J.*, 1940, **34**, 392, 846, 853, and in the press.

strains of *Bact. coli*, *Bact. proteus*, *Cl. welchii*, *Cl. aerofætidum*, *Cl. bifermentans*, decarboxylate glutamic acid to γ -aminobutyric acid. The distribution of the various decarboxylases shows that each enzyme is specific for one amino-acid.

G. M. Hills⁴⁵ has shown that certain *Streptococci* and *Staphylococci* will attack *l*(+)-arginine to produce ornithine by splitting off ammonium carbonate. This reaction is carried out by strains of *Strep. faecalis* and consequently a symbiotic mixture of *Strep. faecalis* and *Bact. coli* attacks arginine in an interesting fashion, the product of the attack at p_{H} 4.0 being agmatine and at p_{H} 5.5, putrescine, produced with ornithine as an intermediate substance.⁴⁶

H. L. A. Tarr⁴⁷ has shown that the trimethylamine in putrid fish is produced by reduction of trimethylamine oxide by bacteria which possess an enzyme activating the trimethylamine oxide so that it can be reduced by any one of a number of dehydrogenase systems. Since only a few of the bacteria infecting putrid fish possess this enzyme, the trimethylamine production cannot be regarded as a measure of putrefaction.

Purine Metabolism.—M. Stephenson and A. R. Trim⁴⁸ have continued the investigations started by C. Lutwak-Mann⁴⁹ on the breakdown of adenylic acid and other adenine compounds by *Bact. coli*. Muscle adenylic acid is deaminated and dephosphorylated by *Bact. coli*, the dephosphorylation appearing to precede deamination. Adenosine is deaminated to inosine and the ribose is split off and fermented; the fermentation of ribose in adenosine is about 10 times as fast as that of free ribose. Adenine is slowly deaminated to hypoxanthine, the rate of deamination being increased some 6—7 times by the presence of adenosine—an effect similar to that found in the deamination of aspartic acid by aspartase II.²⁶ E. F. G.

D. J. BELL.
J. F. DANIELLI.
E. F. GALE.
L. J. HARRIS.
R. HILL.
E. KODICEK.
J. R. MARRACK.
A. NEUBERGER.
F. W. NORRIS.
M. STEPHENSON.

⁴⁵ *Biochem. J.*, 1940, **34**, 1057.

⁴⁶ E. F. Gale, *ibid.*, p. 853.

⁴⁷ *J. Fish. Res. Bd. Can.*, 1939, **4**, 367.

⁴⁸ *Biochem. J.*, 1938, **32**, 1740.

⁴⁹ *Ibid.*, 1936, **30**, 1405.