Kirkwood, S. & Phillips, P. H. (1946). *J. biol. Chem.* **163**, 251.
Kodicek, E. & Worden, A. N. (1945). *Biochem. J.* **39**, 78.
Lilly, V. G. & Leonian, L. H. (1944). *Science*, **99**, 205.
McCance, R. A. & Widdowson, E. M. (1935). *Biochem. J.* **29**, 2694.
McCollum, E. V., Simmonds, N. & Pitz, W. (1916). *J. biol. Chem.* **25**, 105.
McIlwain, H. (1942). *Brit. J. exp. Path.* **23**, 95.
McIlwain, H. & Hawking, F. (1943). *Lancet*, **244**, 449.
Medical Research Council (1924). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 38, 2nd ed.
Mellanby, E. (1921). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 61.
Mellanby, E. (1922). *Brit. med. J.* ii, 849.
Mellanby, E. (1926). *J. Physiol.* **61**, xxiv.
Mellanby, E. (1930). *Brit. med. J.* i, 677.
Mellanby, E. (1931). *Brain*, **54**, 247.
Mellanby, E. (1937). *Toxamins in Food.* In *Perspectives in Biochemistry*, p. 318 [J. Needham and D. E. Green, editors]. Cambridge: University Press.
Melville, D. B., Dittmer, K., Brown, G. B. & Vigneaud, V. du (1943). *Science*, **98**, 497.
Meunier, P. (1948–9). *Brit. J. Nutrit.* **2**, 396.
Meunier, P., Mentzer, C., Buu Hoï & Cagniant (1943). *Bull. Soc. Chim. biol., Paris*, **25**, 384.
Moore, P. H. (1914). *Rep. exp. Fms Can.*, p. 21.
Oleson, J. J., Hutchings, B. L. & Subbarow, Y. (1948). *J. biol. Chem.* **175**, 359.
Ott, W. H. (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 125.
Putney, W. W. (1945). *J. Amer. vet. med. Ass.* **106**, 164.
Quastel, J. H. & Wooldridge, W. R. (1928). *Biochem. J.* **22**, 689.
Quick, A. J. (1937). *Amer. J. Physiol.* **118**, 260.
Radeleff, R. D. (1945). *Vet. Med.* **40**, 280.
Roderick, L. M. (1931). *Amer. J. Physiol.* **96**, 413.
Slade, R. E. (1945). *Chem. & Ind.* **64**, 314.
Snell, E. E., Chan, L., Spiridanoff, S., Way, E. L. & Leake, C. D. (1943). *Science*, **97**, 168.
Stahmann, M. A., Huebner, C. F. & Link, K. P. (1941). *J. biol. Chem.* **138**, 513.
Unna, K. (1943). *Proc. Soc. exp. Biol., N.Y.*, **54**, 55.
West, R. (1941). *Proc. Soc. exp. Biol., N.Y.*, **46**, 369.
West, R. & Coburn, A. F. (1940). *J. exp. Med.* **72**, 91.
Weswig, P. H., Freed, A. M. & Haag, J. R. (1946). *J. biol. Chem.* **165**, 737.
Williams, R. R. (1927). *Biochem. J.* **21**, 1349.
Wood, W. B. Jr. & Austrian, R. (1942). *J. exp. Med.* **75**, 383.
Woods, D. D. (1940). *Brit. J. exp. Path.* **21**, 74.
Woods, D. D. & Fildes, P. (1940). *Chem. & Ind.* **59**, 133.
Woolley, D. W. (1941). *J. biol. Chem.* **141**, 997.
Woolley, D. W. (1944). *J. biol. Chem.* **154**, 31.
Woolley, D. W. (1945*a*). *J. biol. Chem.* **157**, 455.
Woolley, D. W. (1945*b*). *J. biol. Chem.* **159**, 59.
Woolley, D. W. (1946*a*). *J. biol. Chem.* **162**, 179.
Woolley, D. W. (1946*b*). *J. biol. Chem.* **163**, 481.
Woolley, D. W. & Krampitz, L. O. (1943). *J. exp. Med.* **78**, 333.
Woolley, D. W. & Pringle, A. (1948). *J. biol. Chem.* **174**, 327.
Woolley, D. W. & White, A. G. C. (1943). *J. biol. Chem.* **149**, 285.

Anti-B-Vitamins

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The classic concept of vitamin deficiencies, that disease arises solely from the lack of an essential vitamin, has been modified by new developments. Thus, it is now realized that not only the absence of vitamins, but also the presence of 'toxamins' which interfere in some way with the essential nutrient cause symptoms of a deficiency. This concept is not new, having been put forward by Mellanby (1926) more than 20 years ago, but more recently an accumulation of evidence has emphasized its far-reaching

significance. One very important advance has been the discovery by Woods & Fildes (1940) of the antimetabolic activity of structural analogues, as first instanced by the interaction of sulphonamides and *p*-aminobenzoic acid. A complication in the interrelationship between vitamins and toxamins arises from the fact that animals do not rely only on external supplies of certain vitamins but may obtain them from intestinal bacteria living in symbiosis with the host (Elvehjem, 1948).

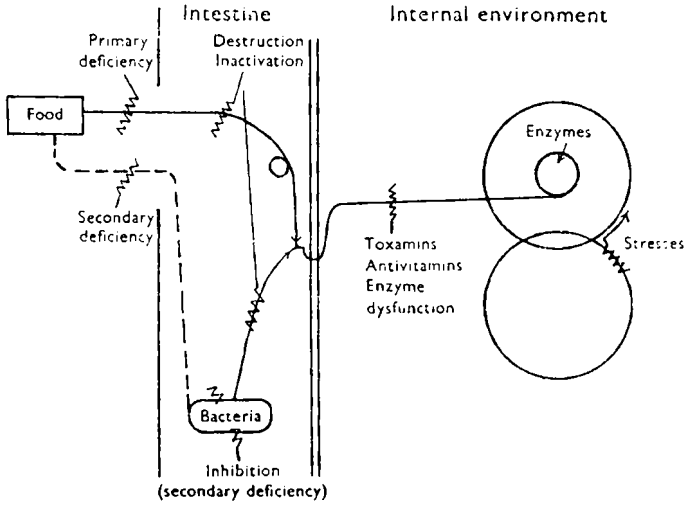


Fig. 1. The normal metabolic pathway of a vitamin and factors interfering with it.

The concept of toxamins and of the intestinal vitamin synthesis has greatly modified the too simple view of the development of deficiencies, which resulted from experiments in which conditions were strictly controlled and only one or two factors varied. Mellanby (1937) said truly that 'the pendulum has swung back from the extreme of the deficiency theory'. Clinicians and workers in the field support the view that the question of deficiency is often more complex than described by experimentalists. Our increasing knowledge of the many factors involved may be of service in bridging the gap between the experiences of workers in the field and in the laboratory.

Let us consider first, the normal metabolic pathway of a vitamin (Fig. 1). It can be supplied in the food, bound or free, it is then liberated in the intestines and has to cross the barrier of the cell wall. It is then incorporated, as the B-vitamins, in enzyme systems which act on a number of metabolic cycles. Another supply of the vitamin, which may be only small, is provided by intestinal bacteria.

Apart from a primary deficiency due to lack of the vitamin many other factors may interfere, and produce similar results. The metabolic pathway can be interrupted in the following ways: (1) certain substances or bacteria may destroy or inactivate the vitamins. Thus Bilharziosis is an important factor in the development of pellagra in Egypt. The avidin-biotin complex may be given as an example of inactivation of a vitamin by a substance which combines with it in the intestines. (2) If bacterial growth is inhibited by the presence of some antagonistic substance or the absence of an essential

metabolite, the bacteria will fail to produce the vitamin, or vitamins, required by the host. (3) The absorption of the vitamin through the intestinal wall may be impaired or prevented. (4) When the vitamin has reached the internal environment it may be displaced by structural analogues from the surface on which it acts. Its action may also be impaired by faulty functioning of the enzymes by which it is incorporated into certain functional systems. (5) When the vitamin is incorporated into enzyme systems, the metabolic cycles in which it is concerned may be interfered with by stresses, such as infections, pregnancy, imbalance of amino-acids, carbohydrates and fats, all of which increase the need for a particular vitamin.

Structural analogues (antivitamins)

I prefer to use the term, antivitamin, only for a structural analogue of the vitamin and to use toxamin as a general term for a substance causing deficiency symptoms. Our extensive knowledge of structural analogues has been reviewed by Welch (1945), Woolley (1946*a*, *b*, 1947), Knight (1945, 1946), Roblin (1946), Ershoff (1948).

A survey of evidence at present available indicates the following points: (1) The antimetabolite must have a structural resemblance to the vitamin. Rydon (1948) suggests that the resemblance must be a spatial one. Erlenmeyer (1948) goes further, maintaining that the analogue should be isosteric with the vitamin. (2) If we assume that the antivitamin acts by displacing the vitamin from active surfaces, the structural change must be in or near the groups which are responsible for its vitamin action, and not in that group which is essential for its attachment to the protein carrier. (3) The inhibiting effect of the antimetabolite must be reversible by the vitamin, and the inhibition index, i.e. the ratio of analogue to vitamin which produces a half-maximum growth, must be constant over various concentrations of vitamin and antivitamin. If competitive inhibition cannot be proved, it may be dangerous to draw conclusions that the inhibitor is an antivitamin.

Mode of action of structural analogues

The mode of action of structural analogues has been extensively studied in recent years. It was first suggested that the antivitamin displaces the vitamin from active protein surfaces on which it is firmly bound as part of the prosthetic group of the enzyme (displacement theory). However, all the experimental evidence points to the view that the competition between the vitamin and antivitamin occurs at an earlier stage, when the vitamin is the substrate of a reaction in which it is modified and fixed to the protein carrier. During this process the antivitamin competes actively with the vitamin, and if conditions are favourable it is fixed instead of the vitamin on the protein surface. Another mode of action may also be visualized, in which activity of the enzyme concerned with the preliminary metabolic transformation of the vitamin may be impaired.

It is evident that these considerations apply only to vitamins which form a part of coenzymes, especially the B-vitamins. Fat-soluble vitamins which most probably act differently or at least act in lipid-water (two-phase) systems and have therefore more

complex thermodynamic conditions, evidently need a modified theory of antivitamin action. However, this has to await further advances in our knowledge of the thermodynamics and mode of action of these vitamins.

Chemical changes producing antivitamin action

According to Woolley (1947) there are three main changes in structure which may produce an active antagonist:

(1) *Replacement of certain radicals:* (*a*) replacement of the carboxyl group in acidic metabolites by sulphonic acid or amide or its derivatives; (*b*) replacement of the carboxyl group by a carboxyalkyl group.

(2) *Interchange of one atom in the ring system of the metabolite:* replacement of sulphur by the vinylene group (—CH=CH—); change of a nitrogen for a carbon, or of a carbon for a nitrogen; replacement of an oxygen by nitrogen.

(3) *Miscellaneous changes.* This group contains changes which cannot yet be classed specifically, such as replacement of a hydroxyl group by an amino group, of a methyl group by chlorine or a change in the side chain, so far as the side chain is an active part of the vitamin molecule.

Use of structural analogues for detection of intermediate compounds in metabolic cycles

The inhibition index has been used in an ingenious way to detect intermediate compounds of a reaction in which the vitamin is concerned as a coenzyme. Thus the inhibition index will be changed if a substance is added which is the substrate of the reaction which the vitamin catalyses as part of an enzyme system. If an intermediate compound is added the amount of inhibitor will have to be increased to obtain a half-maximum inhibition and thus the inhibition index will increase. This 'inhibition analysis' has been introduced by Shive & Macow (1946) and Beerstecher & Shive (1946) to demonstrate which substances are intermediates in a specific reaction in which the vitamin is concerned. For example, thymine and thymidine, the desoxyriboside of thymine, have been shown to be connected with the coenzyme activity of pteroylglutamic acid. A word of caution has been sounded recently by Hitchings, Elion & VanderWerff (1948) who point out that the too generous application of this analysis may result in false conclusions.

TOXAMINS AFFECTING INDIVIDUAL B-VITAMINS

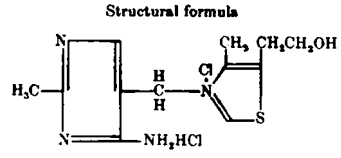
Vitamin B_1

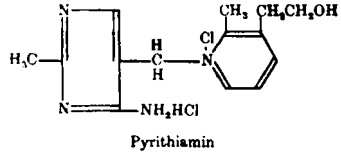
Fig. 2 shows the structural formula of vitamin B_1 and of the structural analogues and other toxamins which may cause an inhibition of the vitamin's action.

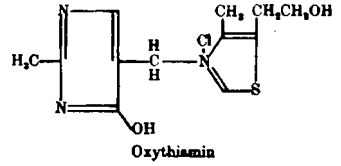
Structural analogues

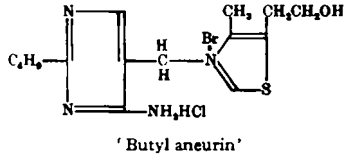
Pyrithiamin. By replacing the sulphur atom in the thiazole part of the molecule by —CH=CH— an isosteric compound is produced which has a pyridine instead of a thiazole nucleus. It was found that this compound is a strong antagonist for animals

and micro-organisms, and that its action can be reversed by aneurin. Only bacteria which require the vitamin were sensitive (Woolley & White, 1943*a*, *b*; Wyss, 1943; Sarett & Cheldelin, 1944; Robbins, 1941; Tatum & Bell, 1946). Not all such bacteria, however, were inhibited; when the thiazole moiety was present in the medium the growth of *Mycobacterium paratuberculosis* was promoted by the pyrimidine part of the molecule (Lutz, 1948).









Toxamin	Species or enzyme affected	Reference
Pyrithiamin	Animals Bacteria	Woolley & White (1943*a*, *b*)
Oxythiamin	Mouse Thiaminase	Soodak & Cerecedo (1944)
'Butyl aneurin'	Rat	Emerson & Southwick (1945)
Thiaminase	Animals	Woolley (1941*a*)
Live yeast	Man	Price, Marquette & Parsons (1947)
Fern factor	Bovine, horse, rat	Weswig, Freed & Haag (1946)

Fig. 2. Vitamin B_1 and its antagonists.

Oxythiamin. If the amino group at position 4 of the pyrimidine is replaced by a hydroxyl group, an active antagonist is produced, which gives deficiency symptoms in mice. The analogue inhibits also the Chastek paralysis factor, and in this respect supports the action of the vitamin (Soodak & Cerecedo, 1944).

n-*Butyl homologue of aneurin* (*'butyl aneurin'*). If the methyl group in the pyrimidine ring is replaced by a butyl radical an active antagonist is produced, capable of causing in rats signs of vitamin deficiency which can be reversed by vitamin B_1 (Emerson & Southwick, 1945). This is an interesting phenomenon, since inhibition is not caused by substitution with groups of less than four carbon atoms. Thus the ethyl homologue still has vitamin B_1 activity, whereas the *n*-propyl homologue has little activity as either a vitamin or an antivitamin.

Other toxic factors

Thiaminase. The Chastek paralysis factor toxic for foxes which was found to be present in the raw flesh of carp (Green, Carlson & Evans, 1941) has been found to be an enzyme which destroys aneurin (Woolley, 1941*a*). It is also present in the Atlantic herring (*Cluper harengus*) and in clams, shrimps and some mussels (Deutsch & Hasler, 1943; Yudkin, 1945; Melnick, Hochberg & Oser, 1945; Neilands, 1947; Jacobsohn & Azevedo, 1947). Its occurrence in certain fish which may be eaten by man in a raw or partly cooked state necessitates caution when these foods are included in the diet. Raw fish produced clinical symptoms, similar to Wernicke's encephalopathy due to vitamin B_1 deficiency, in foxes, cats, rats and young pigeons (Evans, Carlson & Green, 1942; Smith & Proutt, 1944; Abderhalden, 1947). The enzyme has been purified by Ågren (1946), and can be divided by dialysis into a heat labile apo- and a heat stable co-ferment (Krampitz & Woolley, 1944). Thiaminase splits vitamin B_1 into two parts at the methylene bridge. It seems to be unique in its enzyme action in that it produces hydrogen ions during the cleavage of the vitamin B_1 molecule (Sealock & Livermore, 1944).

Bracken-fern factor. A plant factor from bracken fern (*Pteris aquilina*) has been isolated which causes signs of vitamin B_1 deficiency in rats and seems to be responsible for a disease occurring in cattle and horses feeding on fern pasture. It withstands heat, is insoluble in ethyl ether and acetone and slightly soluble in 92% ethanol. Other related species of plants do not contain appreciable amounts of it. It is contained in air-dried fern but not in fern dried in air and sun, nor in fern steamed for 30 min. (Weswig, Freed & Haag, 1946; Haag, Weswig & Freed, 1947; Haag & Weswig, 1948).

Other possible toxamins have been described in grain (Hart, McCollum & Steenbock, 1911; Hart, Miller & McCollum, 1916; Moore, 1914; McCollum, Simmonds & Pitz, 1916; Williams, 1927; Bhagvat & Devi, 1944) and in raw mutton (Radeleff, 1945; Putney, 1945), but further study will be necessary before they can be accepted as true vitamin antagonists.

Effect of live yeast. Another factor which may interfere with the normal supply of aneurin in man has been recently described. According to Price, Marquette & Parsons (1947) and Kingsley & Parson (1947) live yeast may interfere with the normal supply of aneurin in man. These workers found that its ingestion did not result in an increased urinary excretion of vitamin B_1 and riboflavin, but that the concentration of both factors in faeces was increased. It appears, therefore, that not only are the vitamins of live yeast little absorbed, but the yeast itself competes for the vitamins with the host. Control studies with dried dead yeast confirmed this conclusion.

Other forms of antagonist, acting on enzymes

Thiazole pyrophosphate. The pyrophosphate of thiazole, but not the monophosphate or thiazole alone, inhibits the activity of cocarboxylase in yeast. This antagonism was reversed only by cocarboxylase. Buchman, Heegaard & Bonner (1940) suggest that this is due to a competition of the antagonists with cocarboxylase for the enzyme protein. Similarly, sulphathiazole, bisulphite and a number of quinones inhibit the

activity of carboxylase (Sevag, Shelburne & Mudd, 1942; Wallerstein & Stern, 1945; Kuhn & Beinert, 1947). Some of these compounds may react by forming a bisulphite-pyruvate complex and thus eliminating the substrate or, as the quinones, may react with one or more thiol groups of the enzyme protein. These inhibitors may eventually produce defects of metabolism which resemble those occurring in a deficiency.

Inhibitor of thiaminase. Substitution of the pyridine ring by an *o*-aminobenzyl group, i.e. substitution of two nitrogens by two carbons and loss of side groups and the omission of the hydroxyethyl side chain of the thiazole produces an inhibitor of thiaminase, 3-(*o*-aminobenzyl)-4-methylthiazolium chloride (Sealock & Goodland, 1944). This compound can also be regarded as a supporter of vitamin B_1 preventing its destruction by the enzyme. It seems to act by competing with aneurin for the thiaminase.

Changes in the molecule which result in inactivation

As a point of interest, compounds may be mentioned in which the sulphur in thiazole has been replaced either by selenium or an imino group. Whereas the former compound has no vitamin activity (Schultz, 1940), the latter inhibits *Lactobacillus fermenti* when added in high concentrations (Erlenmeyer, Waldi & Sorkin, 1948).

Replacement of the β-hydroxyethyl side chain of the thiazole at position 3 by another radical results in loss of activity (Buchman & Richardson, 1945). Loss of activity also results if the amino group in position 4′ of the pyrimidine ring, or if the methyl group in position 2 of the thiazole, is replaced, and also if the methylene bridge between the thiazole and pyrimidine is modified (Bergel & Todd, 1937).

Conclusions

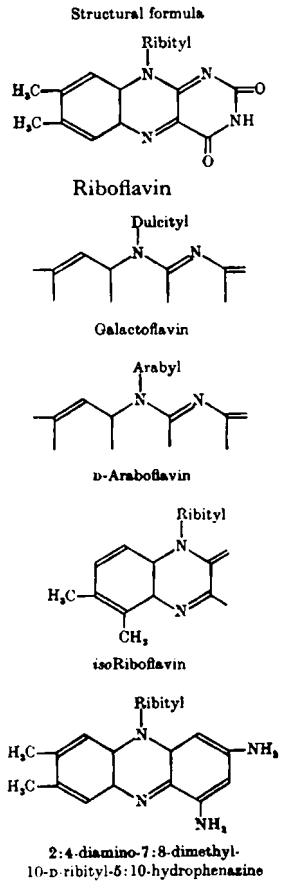
Only a few factors have been found in nature which antagonize vitamin B_1. It has still to be seen if some of them bear any similarity to synthetic structural analogues. The study of the synthetic compounds has added to the knowledge of the active groups in the vitamin molecule. As a change in the hydroxyethyl side chain of the thiazole results in a definite loss of vitamin activity, whereas substitution of other groups produces antivitamins, it is reasonable to assume that the link with the active protein surface is effected at this point through a pyrophosphate link. There are of course other factors which affect the metabolism of vitamin B_1 such as imbalance of carbohydrates, fats and protein, infections and other physiological stresses, and impairment of microbiological synthesis of the vitamin, which all come under the wider scope of antagonism.

Riboflavin

No naturally occurring antagonist has been found, but several synthetic analogues have proved efficient antagonists in animals and bacteria. Fig. 3 shows the formula of riboflavin, [6:7-dimethyl-9-(D-1′-ribityl)*iso*alloxazine], and the substitutions which have been made. They have all been described in the reviews mentioned on p. 375 and warrant, therefore, only a passing reference.

Substitution of the ribityl side chain by a dulcityl produces galactoflavin which is an antagonist for rats (Emerson, Wurtz & Johnson, 1945). Inversion of the position of the

hydroxyl groups in the side chain produces D-araboflavin which also proves to be a vitamin antagonist in rats (Euler & Karrer, 1946). A shift in the position of the methyl groups from 6:7 to 7:8 produced *iso*riboflavin which was found to be an efficient antivitamin in animals, but not for bacteria (Emerson & Tishler, 1944; Foster, 1944). Another structural analogue is the 2:4-diamino-7:8-dimethyl-10-D-ribityl-5:10-dihydro-



Toxamin	Animal affected	Reference
	Rat	Emerson, Wurtz & Johnson (1945)
	Rat	Euler & Karrer (1946)
	Rat	Emerson & Tishler (1944)
	Mouse	Woolley (1944)

Fig. 3. Riboflavin and its antagonists.

phenazine (Woolley, 1944; Sarett, 1946) which is an antagonist for micro-organisms and mice. The change in the structure, as will be seen, consists in a substitution of two nitrogens of the pyrimidine ring by two carbons and the substitution of two hydroxyl groups by two amino groups. If the ribityl side chain is replaced by a methyl group (lumiflavin) the compound inhibits the growth of *Lb. casei* in the presence of riboflavin (Sarett, 1946). The substitution of the two methyl groups in the 6 and 7 positions by chlorine atoms produces dichlororiboflavin which is bacteriostatic (Kuhn, Weygand & Möller, 1943). Schopfer (1948) found that an organism autotrophic for riboflavin, *Eremothecium Ashbyii*, was inhibited by dichlororiboflavin and he suggests that this cannot be explained by the displacement theory or by competition with riboflavin, since the organism continued to produce riboflavin.

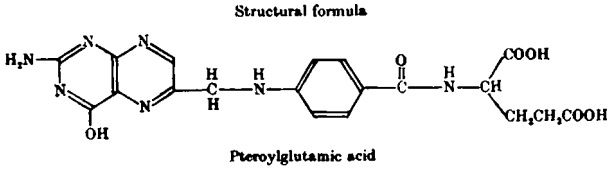
Conclusions

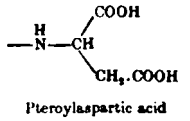
The study of structural analogues has shown the importance for the activity of riboflavin of the ribityl side chain, of the two methyl groups and of the pyrimidine in the *iso*alloxazine ring.

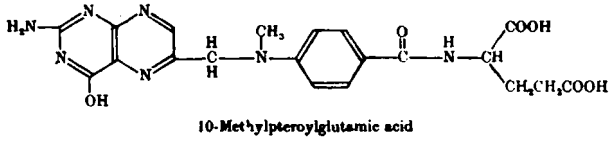
The ribityl side chain links up to form the dinucleotides. If the hypothesis mentioned on p. 375 is correct, the attachment to the protein carriers may be either at the nitrogen in position 5, or at some other part of the dinucleotide molecule other than the *iso*-alloxazine moiety.

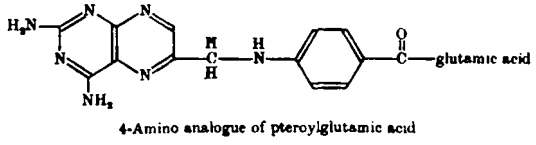
Pteroylglutamic acid (PGA)

Fig. 4 shows the structural formula of PGA, N-[4-{[(2-amino-4-hydroxy-6-pteridyl)-methyl]-amino}-benzoyl]-D-glutamic acid, and of the structural analogues which have been studied in recent years. They can be roughly divided into the following groups:









	Toxamin Animal affected	Reference
	Chick	Hutchings, Mowat, Oleson Stokstad, Boothe, Waller, Angier, Semb & Subbarow (1947)
	Rat Chick Mouse Pig	Franklin, Stokstad & Jukes (1947, 1948); Franklin, Stokstad, Belt & Jukes (1947)
	Rat Chick	Franklin, Stokstad & Jukes (1948)

Fig. 4. Pteroylglutamic acid and its antagonists.

Glutamic acid replaced by another amino-acid

Pteroylaspartic acid has been synthesized by Hutchings, Mowat, Oleson, Stokstad, Boothe, Waller, Angier, Semb & Subbarow (1947) and by Woolley & Pringle (1948). This compound is an efficient antagonist for *Lb. casei*, *Streptococcus faecalis* R and, to a lesser degree, for the chick. It was inactive for rats and for *Bacterium coli*. Its action was reversed by PGA and also by thymine.

Methylfolic acid (*10-methylpterin*)

Much more potent inhibitors were synthesized by Martin, Tolman & Moss (1947) and by workers in the Lederle Laboratories. Addition of a methyl group at position 10, i.e. on the amino group of the pteroic acid part of the molecule gave a potent inhibitor for bacteria and rats. Franklin, Stokstad, Belt & Jukes (1947) prepared a crude antagonist in which L-glutamic acid was used for synthesis instead of the D-glutamic acid which Martin *et al.* (1947) used. The inhibitor was more active for *Strep. faecalis* R than for *Lb. casei* (inhibition index 20 and 1000, respectively). Adenine and thymine together, though not separately inactivated that antagonist. Rats, chicks and mice were sensitive to the antagonist and PGA reversed its action. Thymine, however, was inactive (Stokstad, Regan, Franklin & Jukes, 1948; Franklin, Stokstad & Jukes, 1947). Anaemia resulting from the antagonistic action of crude methylfolic acid was observed in a pig. The condition was improved by the administration of gastric juice and by a crude-casein diet which did not contain folic acid (Welch, Heinle, Sharpe, George & Epstein, 1947). A pure *N*-10-methylfolic acid and a *N*-10-methylpteroic acid have been recently prepared by Cosulich & Smith (1948).

Methylfolic acid and also the aspartic acid analogue of PGA inhibited the dopa decarboxylase. PGA reversed the inhibition. On the other hand, the tyrosine decarboxylase was not affected (Martin & Beiler, 1947). The action of methylfolic acid was counteracted by thymidine, the desoxyriboside of thymine. This led Shive, Eakin, Harding, Ravel & Sutherland (1948) to suggest that thymidine is the end substrate in the synthesis of which PGA is concerned as part of an enzyme system.

4-*Aminopteroylglutamic acid* (4-*aminopterin*)

The most potent vitamin inhibitor was synthesized recently by Seeger, Smith & Hultquist (1947). The L-glutamic analogue was more effective than the D-glutamic. The inhibition index for *Strep. faecalis* R was only 25. Unfortunately the compound proved very toxic, especially for animals. Thus mice, chicks and rats developed symptoms resembling folic acid deficiency but PGA reversed the antagonistic action only partly, and then only at the lowest active concentration of inhibitor used (Franklin *et al.* 1948; Oleson, Hutchings & Subbarow, 1948; Swendseid, Wittle, Moersch, Bird & Brown, 1948). The inhibition by 4-aminopterin was not strictly of a competitive nature, and Oleson *et al.* (1948) mention that it is difficult to decide if the antagonistic action is due to the production of a deficiency or to a toxic pharmacological effect. The reversal by PGA seems to point rather to the former possibility.

The change in the structure which produced this antagonist was very minor, namely the replacement of the hydroxyl group at position 4 by an amino group. Further progress is being made in testing less toxic derivatives. Thus 4-amino-10-methylpterin was synthesized, ten times less toxic than the original antagonist (Smith, Cosulich, Hultquist & Seeger, 1948). Other possible analogues which are being tested now are the 4-amino-10-methylpteroic acid, 4-aminopteroylaspartic acid and the 4-aminopterin analogues with sulphanilamide (the carbonyl group replaced by a sulphonyl).

The aminopterins have aroused particular interest in the United States where the Lederle Laboratories and the Sloane-Kettering Institute are investigating their effect in inhibiting neoplastic growth (cited by Woolley, 1948*a* and Dodds, 1948).

Anticarcinogenic activity of analogues. Woll (1948) found that Rous chicken sarcoma treated with vitamin analogues of PGA regressed completely or partly. Similar findings are reported by Little, Sampath, Paganelli, Locke & Subbarow (1948). They suggest that the inhibition of the growth of Rous sarcoma by 4-aminopterin (with L-glutamic acid) may be due to inhibition of the multiplication of the virus. This is of interest in view of the observation of Bauer (1948) that the activity of xanthine oxidase is connected with the multiplication of viruses. As will be mentioned later certain analogues of PGA effectively inhibit xanthine oxidase (Kalckar, Kjeldgaard & Klenow, 1948). Certain improvements in the course of acute leukaemia were reported recently by Meyer (1948), by Farber, Diamond, Mercer, Sylvester & Wolff (1948) and in Editorial (1948). Spontaneous improvements cannot be excluded, especially as the therapeutic results were only temporary remissions. Nevertheless, the remission rate in the series of Farber *et al.* (1948) of ten out of sixteen cases of acute and subacute leukaemia in children is far beyond the spontaneous rate of remission. The series of sixteen adults and two children mentioned in Editorial (1948) is less convincing with four and one remissions, respectively. The antivitamin proved highly toxic, producing such reactions as haemorrhage and aplastic anaemia. But if one takes into account the private reports of yet unpublished work of the American teams, important developments may soon be expected.

Other 2:4-diaminopterin derivatives were prepared by Daniel, Norris, Scott & Heuser (1947) and Daniel & Norris (1947). They are the 2:4-diamino-6:7-dimethylpteridines and the -6:7-diphenylpteridines which proved to be antagonists for bacteria, whether or not they required preformed PGA for their normal growth.

Inhibition of xanthine oxidase by 6-pteridylaldehyde

This has recently been reported by Kalckar *et al.* (1948); pteridine oxidase was also inhibited. This finding reveals a further link between PGA and the metabolism of purines. The action of this antagonist and its reversal by PGA must certainly have an effect on the oxidative breakdown of purines. Such a connexion between the metabolism of purines and PGA was suggested some time ago. The discovery by Woods (1940) of the antagonistic action of sulphonamides and *p*-aminobenzoic acid (PAB) is amplified by the finding of PAB in the molecule of PGA. Stokes (1944) suggested that PGA forms a prosthetic group of an enzyme concerned in the synthesis of thymine. The work of Lampen & Jones (1946, 1947) and of Woods (1948) points in the same direction. The observation of Shive *et al.* (1948) that thymidine may be the end product of this reaction further confirms this hypothesis. Thymidine is required by a number of lactobacilli which formerly could not be grown on simple synthetic media (Snell, Kitay & McNutt, 1948).

Quinoxaline-2-carboxylyl-p-*aminobenzoylglutamic acid*

The replacement of the pyrimidine ring by a benzene ring produces an antagonist for riboflavin. This led Woolley & Pringle (1948) to synthesize a similar structural analogue for PGA. The synthesis proved difficult and therefore another similar compound was synthesized in which the methylene bridge was replaced by a carbonyl group. This inhibitor was active only for *Lb. casei* but was inactive for other bacteria or animals.

Iso*xanthopterin*-p-*aminobenzoylglutamic acid* (2-*amino*-4:7-*dihydroxypteridine*-6-*carboxyl*-p-*aminobenzoylglutamic acid*)

Another compound having antagonistic activity was synthesized by Woolley & Pringle (1948) from *iso*xanthopterincarboxylic acid. The main difference from PGA is in the replacement of the methylene bridge by a carbonyl group and substitution of one hydrogen by a hydroxyl group at position 7 of the pteridine ring. The compound was slightly more active than the one mentioned above, with an inhibition index of 7000. Bacteria requiring PGA were inhibited and PGA reversed the inhibition. The compound was also active as an inhibitor for rats in amounts of 10 mg. daily.

Finally one may mention the antagonistic effect of sulphonamides which has been fully utilized in nutritional work for the development of folic acid deficiency in animals.

Conclusions

From these substitution studies it is clear that the hydroxyl group in position 4, the methylidine group at position 7, the CH_2-NH bridge at positions 9 and 10, and the entire *p*-aminobenzoylglutamic acid side chain are active groupings essential for the vitamin activity. The amino group in position 2 is essential, and loss of biological activity follows its replacement by an hydroxyl group (Daniel *et al.* 1947). This group is common to all active compounds, having a vitamin or antivitamin effect, and it is possibly the point of attachment to the protein carrier or other active surface.

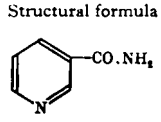
Nicotinamide

Fig. 5 shows the structural formula of nicotinamide and corresponding structural analogues which were found antagonistic.

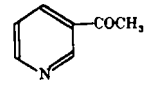
Replacement of the functional carboxyl or carboxyamide group by sulphonic acid or by an acetyl group (pyridine-3-sulphonic acid and 3-acetylpyridine) led to antagonists active for bacteria and animals. The former was active for certain micro-organisms (McIlwain, 1940*a*, *b*; Matti, Nitti, Morel & Lwoff, 1941; Möller & Birkofer, 1942; Erlenmeyer & Würgler, 1942), for dogs and rats, but not for mice (Woolley, Strong, Madden & Elvehjem, 1938; Woolley & White, 1943*c*; Krehl, Henderson, de la Huerga & Elvehjem, 1946). 3-Acetylpyridine was an effective antivitamin for mice, but not for bacteria (Woolley, 1945; Auhagen, 1942). Replacement of the vinylene group (—CH=CH—) by sulphur produced a thiazolecarboxyamide bacteriostatic for certain bacteria (Erlenmeyer, Bloch & Kiefer, 1942). These antagonists have not been so extensively studied as those for other vitamins.

Another development of research on nicotinic acid supports the toxamin concept of vitamin deficiencies. Krehl, Teply & Elvehjem (1945) found that rats fed on a low-

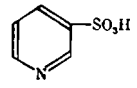
protein, low-tryptophan diet stopped growing if at least 40% of the diet was replaced by maize. Further studies from the Wisconsin laboratory showed that a similar effect could be produced by mixtures of amino-acids (Krehl, Sarma, Teply & Elvehjem, 1946). The inhibition could be reversed by nicotinic acid and by tryptophan which, however, had to be given in much greater amounts, 1·5 and 50 mg., respectively. This finding revealed another important correlation which will be discussed later. Elvehjem's group



Nicotinamide



3-acetylpyridine



3-pyridine sulphonic acid

Toxamin	Animal affected	Reference
	Mouse, not bacteria	Woolley (1945)
	Dog, not mouse	Woolley & White (1943*c*)
Amino-acids: threonine phenylalanine	Rat, dog	Hankes, Henderson, Brickson & Elvehjem (1948)
Maize toxamin	Rat, mouse	Woolley (1946*c*, *d*)
Indole derivatives	Bacteria	Dubos (1946)

Fig. 5. Nicotinamide and its antagonists.

suggested that the antagonistic action of rations low in tryptophan and in nicotinic acid was due to an imbalance of amino-acids affecting the synthesis of nicotinic acid in the gut by micro-organisms. Hankes, Henderson, Brickson & Elvehjem (1948) showed quite recently that DL-threonine or DL-phenylalanine in amounts equivalent to that present in 2% acid-hydrolysed casein, which by itself produces pellagra in rats (Henderson, Deodhar, Krehl & Elvehjem, 1947), aggravated the nicotinic acid-tryptophan deficiency. All other amino-acids fed at comparable levels were without effect. Thus the presence of certain amino-acids in the food in the complete or partial absence of tryptophan and nicotinic acid contributes to the deficiency of nicotinic acid.

At the time when the Wisconsin workers were studying the effects of the imbalance of amino-acids, Woolley was working on the isolation of a toxamin in maize which produced deficiency symptoms in mice (Woolley, 1945, 1946*c*, *d*) and obtained a concentrate active in amounts of 1 mg./100 g. of diet. The substance seems to be of basic nature and further purification is proceeding satisfactorily (Woolley, 1948*c*). Woolley's working hypothesis is that the toxamin may be a pyridine compound possibly related to 3-acetylpyridine. Borrow, Fowden, Stedman, Waterlow & Webb (1948) found that maize bran was particularly effective in producing a nicotinic acid deficiency in rats.

The possible connexion between the pellagrogenic effect of maize and toxamins

related to tryptophan metabolism was studied in Cambridge. We were struck by the high concentration in maize of indole-3-acetic acid (Haagen-Smit, Leech & Bergner, 1942; Berger & Avery, 1944). The first results with an old sample of indoleacetic acid were encouraging, but later we were unable to obtain signs of deficiency in rats with fresh supplies of the acid (Kodicek, Carpenter & Harris, 1946, 1947). Similar negative results were obtained by Krehl, Carvalko & Cowgill (1947) and by Rosen & Perlzweig (1947). We are unable to explain the cause of these discrepancies. One possibility, as we suggested, could be that in our first experiments the microflora of the rats was sensitive to indole-3-acetic acid but that it changed in the course of further experiments. Another possibility may be that the first sample of indoleacetic acid, which was an old preparation, was contaminated with a breakdown product possessed of antitryptophan activity. Bacteriological studies have shown that indole derivatives may interfere with the tryptophan-nicotinic acid metabolism of bacteria (Fildes, 1941; Dubos, 1946; Perlman, 1946).

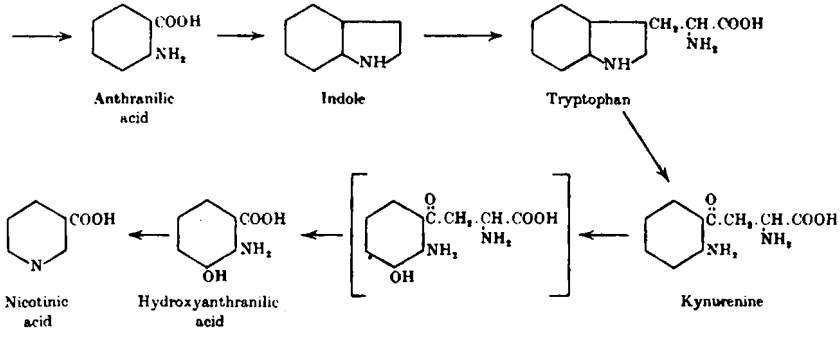


Fig. 6. Possible scheme of biosynthesis of nicotinic acid from tryptophan.

The relation of nicotinic acid to tryptophan and the possibility of tryptophan being a precursor of nicotinic acid has been intensively studied in the United States. Beadle, Mitchell & Nyc (1947), Mitchell & Nyc (1948), Nyc & Mitchell (1948), Bonner (1948) have shown with *Neurospora* mutants that the biosynthesis of nicotinic acid from tryptophan may proceed through kynurenine and 3-hydroxyanthranilic acid (2-amino-3-hydroxybenzoic acid) (Fig. 6).

Mitchell, Nyc & Owen (1948) reported recently that 3-hydroxyanthranilic acid (1 mg./100 g. of diet) had a definite nicotinic acid activity for rats made deficient by a maize diet. The excretion of nicotinic acid and of *N'*-methylnicotinamide increased in normal rats dosed with this substance as after dosing with tryptophan or nicotinic acid (Albert, Scheer & Deuel, 1948). Against this finding stand, however, the results of Bonner (1948) who could not replace nicotinic acid by 3-hydroxyanthranilic acid for the growth of rats. The feeding of radioactive DL-tryptophan-β-C^{14} did not result in excretion of radioactive *N'*-methylnicotinamide, indicating that kynurenic acid (4-hydroxyquinoline-2-carboxylic acid) or its pyridine nucleus, is not the precursor of nicotinic acid. This does not, however, rule out kynurenine and 3-hydroxyanthranilic acid (Heidelberger, Gullberg, Morgan & Lepkovsky, 1948).

Biotin

The antagonists which have been prepared were fully described by Knight (1946) and will be mentioned here only briefly. Fig. 7 shows the structure of biotin and of its analogues.

Analogues. (1) In desthiobiotin two hydrogens replace the sulphur in the biotin ring. This compound is an efficient antagonist for *Lb. casei* but a growth-producing substance for yeast (Dittmer, Melville & du Vigneaud, 1944; Lilley & Leonian, 1944). (2) In biotin sulphone a sulphonyl group replaces the sulphur atom of biotin (Dittmer & du Vigneaud, 1944). (3) 4-Imidazolidone-2-caproic acid is similar to desthiobiotin, but one methyl group has been omitted. It is a weak antagonist for yeast and *Lb. casei*.

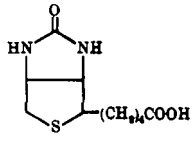
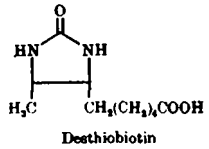
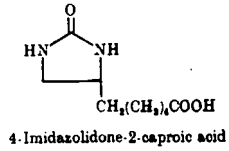
Structural formula	Toxamin		
		Species affected	Reference
 Biotin	Avidin	Rat, dog	Woolley & Longsworth (1942)
		Man	Sydenstricker, Singal, Briggs, DeVaughn & Isbell (1942)
 Desthiobiotin		Bacteria	Dittmer, Melville & du Vigneaud (1944)
 4-Imidazolidone-2-caproic acid		Bacteria	Dittmer, Melville & du Vigneaud (1944)

Fig. 7. Biotin and its antagonists.

This and the other compounds can replace biotin in the biotin-avidin complex but only if the urea group of the biotin molecule is intact, which suggests that this is possibly the grouping at which biotin is attached to avidin (Dittmer & du Vigneaud, 1944; Rogers & Shive, 1947). (4) ω-2:3-Ureylen*cyclo*hexyl aliphatic acids were produced by replacing the sulphur atom by two methylene groups. These are antagonists for yeast and *Lb. casei* which can also combine with avidin (English, Clapp, Cole, Halverstadt, Lampen & Roblin, 1945).

Avidin. The connexion between biotin and avidin was reviewed by Hertz (1946). Eakin, Snell & Williams (1941) and Woolley & Longsworth (1942) isolated from egg white a protein, called avidin, which formed a stable complex with biotin in strict stoichiometric relation. Avidin combines with biotin in the intestinal tract of animals and of man and thus makes the vitamin unavailable for the host, so that biotin deficiency results. Sydenstricker, Singal, Briggs, DeVaughn & Isbell (1942) claim to have obtained deficiency symptoms in volunteers by incorporating daily 200 g. of dehydrated

egg white in their diet; but Rhoads & Abels (1943) and Kaplan (1944) were unable to confirm this finding. Recently, however, Oppel (1948) found a decreased excretion of biotin after ingestion of avidin.

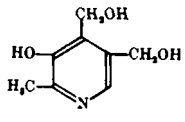
Effect of sulphonamides. The effect of sulphonamides on the bacterial flora of the intestines of experimental animals proves a useful tool in the production of biotin deficiency. Oppel (1948) found a decrease in biotin excretion in man after ingestion of succinylsulphathiazole. A sulphonic acid analogue of desthiobiotin, 4-methyl-5-(ϵ-sulphoamyl)-2-imidazolidone, which inhibits the growth of yeast but not of *Lb. casei*, has been described by Duschinsky & Rubin (1948).

Vitamin B_6

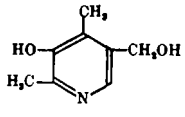
Fig. 8 shows the structural formula of pyridoxin (2-methyl-3-hydroxy-4:5-dihydroxymethylpyridine) and of the structural analogues which inactivate it.

Desoxypyridoxin. Substitution of an hydroxyl group by hydrogen in the hydroxymethyl group at position 4 of the pyridine ring gives desoxypyridoxin (Ott, 1946). This compound is antagonistic for chicks, mice, rats and monkeys (Stoerk & Pappenheimer, 1948; Nelson & Evans, 1948; Emerson, 1947; Mushett, Stebbings & Barton, 1947). It has, on the other hand a slight pyridoxin activity for *Lb. plantarum* (Möller, Zima, Jung & Moll, 1939).

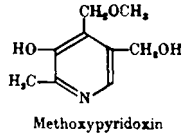
Structural formula



Pyridoxin



Desoxypyridoxin



Methoxypyridoxin

Toxamin	
Animal affected	Reference
Chick	Ott (1946)
Rat, mouse	Stoerk & Pappenheimer (1948)
Monkey	Mushett, Stebbins & Barton
Dog	(1947)
Chick	
Dog	Mushett *et al.* (1947)
Monkey	

Fig. 8. Pyridoxin and its antagonists.

Administration of this analogue produced a severe deficiency with an atrophy of normal and neoplastic lymphoid tissue, microcytic anaemia and atrophy of the spleen. These findings are very similar to those in vitamin B_6 deficiency observed by Carpenter, Harris & Kodicek (1948–9) and Carpenter & Kodicek (1948–9). A decrease in the number of lymphocytes with a relative increase of granulocytes has been found. As in vitamin B_6 deficiency produced by a deficient diet, desoxypyridoxin caused an increased excretion of xanthurenic acid and kynurenine (Porter, Clark & Silber, 1947). In enzyme studies Beiler & Martin (1947) found that desoxypyridoxin is ineffective as an inhibitor for tyrosine decarboxylase but is an effective inhibitor once it is phosphorylated.

Methoxypyridoxin. Ott (1946) and Mushett *et al.* (1947) described another analogue in which the hydrogen of the hydroxymethyl group at position 4 was replaced by a methyl group. This compound proved to be as effective as desoxypyridoxin.

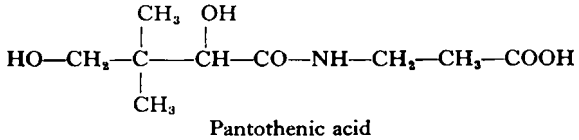
Other derivatives. Further derivatives, such as 2-methyl-3-hydroxy-4-hydroxymethylpyridine and 2-ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine, have been prepared (Martin, Avakian & Moss, 1948). These proved to be even more powerful antagonists for *Saccharomyces cerevisiae.*

If the position 4 of the pyridine ring was occupied by alkylamines, active growth factors were produced (Heyl, Luz, Harris & Folkers, 1948). β-Phenylethylamine, tyramine, tryptamine and benzylamine were equally effective in these substituted pyridoxylamines.

The bacterial flora may contribute to the amount of vitamin B_6 available to the organism as was shown by Carpenter *et al.* (1948). Insoluble sulphonamide in the diet enhanced the onset of vitamin B_6 deficiency in experimental animals. An increase in the proportion of protein in the diet also quickens the onset of the deficiency by increasing the requirement of the organism for vitamin B_6 (Cerecedo & Foy, 1944).

Pantothenic acid

The structural formula of pantothenic acid is shown below. A number of structural analogues proved active antagonists but mainly for micro-organisms.



Pantothenic acid
(αγ-dihydroxy-ββ-dimethyl-butyryl-β-alanide)

Thus by replacement of the carboxyl group by SO_3H pantoyltaurine was obtained (Snell, 1941). It is an effective antagonist for bacteria, but not for animals. A preliminary study by Snell, Chan, Spiridanoff, Way & Leake (1943) indicated that the feeding of relatively large amounts of this analogue to mice produced apparent signs of deficiency of pantothenic acid. Unna (1943), however, could not confirm this observation. The relative insensitivity of animals led McIlwain & Hawking (1943) to suggest pantoyltaurine as a possible anti-infective agent in vivo, but it was difficult to obtain the concentration in blood necessary for bacteriostatic activity. Recently, Winterbottom, Clapp, Miller, English & Roblin (1947) used certain derivatives of pantoyltaurine successfully against malarial infection in mice and chicks.

Replacement of hydrogen by a methyl group in the α or β position resulted in α- or β-methylpantothenic acid (Pollack, 1943). Another change in the β-alanide portion of the molecule, the replacement of a carboxyl group by a benzoyl group produced phenylpantothenone (Woolley & Collyer, 1945). Replacement of the carboxyl group by hydroxymethyl provided another analogue, pantothenyl alcohol. This compound, though an antivitamin for bacteria, had vitamin activity for rats (Pfaltz, 1943) and possibly also for man on the evidence of the increased amount of pantothenic acid excreted in urine (Rubin, Cooperman, Moore & Scheiner, 1948). These compounds

proved useful in the study of the metabolism of pantothenic acid. Shive, Ackerman, Ravel & Sutherland (1947) concluded from their 'inhibition analysis' that pantothenic acid is concerned with the metabolism of the acids in the Krebs cycle. These and other findings will have to be correlated with the finding that pantothenic acid is part of coenzyme A for the enzyme acetylase (Lipmann, Kaplan, Novelli, Tuttle & Guirard, 1947). Experiments with rats showed that an increase in the protein content of the diet has a sparing effect on the vitamin (Nelson & Evans, 1947).

p-*Aminobenzoic acid and sulphonamides*

Since the original discovery of Woods & Fildes (1940), and of Woods (1940) of the structural relationship between sulphonamides and PAB many studies of the metabolism of bacteria have been published. As already mentioned, PAB seems to function mainly as a constituent of the PGA molecule, but experiments with sulphonamides have shown that it is concerned with the metabolism of thymine, purines and possibly of methionine (Woods, 1948; Lampen & Jones, 1946, 1947; Shive & Roberts, 1946). The sequence of the biosynthesis of PGA is not necessarily the same for all organisms. Thus in some, pteroic acid which contains only PAB and the pteridine ring, is first formed. This may explain the finding of Williams (1944) that the entire *p*-aminobenzoylglutamic acid side chain did not reverse the action of sulphonamides, whereas PAB was effective. The use of sulphonamides in nutritional studies has proved of great value. In Fig. 9 are shown comparative growth curves for rats maintained on diets deficient in single vitamins, with and without sulphonamides. It will be seen that in the presence of succinylsulphathiazole more severe symptoms of deficiency of vitamin B_6, riboflavin and pteroylglutamic acid were produced.

Strepogenin

Woolley (1941*b*) and Sprince & Woolley (1945) described a new growth factor, not yet isolated, belonging to the vitamin B group. It is necessary for the growth of *Lb. casei*, mice, rats, and possibly guinea-pigs.

Recently Woolley (1946*e*) reported that lycomarasmin, the tomato-wilting agent from the pathogenic fungus *Fusarium lycopersici* (Plattner & Clausen-Kaas, 1945*a*, *b*), is an effective antistrepogenin. The pure or crude compound inhibited the strepogenin effect in *Lb. casei*, and the wilting of tomato leaves could be effectively prevented by strepogenin concentrates. Plattner & Clausen-Kaas claimed that lycomarasmin (formula $C_9H_{15}O_7N_3$) yielded on hydrolysis aspartic acid, glycine and pyruvic acid. Woolley (1946*e*) recalling the antagonism between glutamic and aspartic acid which, according to Sprince & Woolley (1944), occurs at the end of the peptide chain of strepogenin proceeded to synthesize serylglycylaspartic acid and glycylserylaspartic acid. These two compounds proved to have an antivitamin activity similar to that of lycomarasmin, but from their low activity and solubility in water he concluded that they were not identical with the toxamin. He also analysed pure lycomarasmin (Woolley, 1948*b*) and suggested a structural formula for this naturally occurring antivitamin containing in addition to glycine and asparagine a new amino-acid, α-hydroxyalanine.

Meso-inositol

Kirkwood & Phillips (1946) found that γ-hexachloro*cyclo*hexane (the insecticide, Gammexane, of Imperial Chemical Industries Ltd.) inhibited the growth of yeast and that the inhibition could be reversed by inositol. The similarity of the compound to inositol led them to suggest that Gammexane acts as an antivitamin for inositol. Further work on this compound has been reported by Buston, Jacobs & Goldstein (1946).

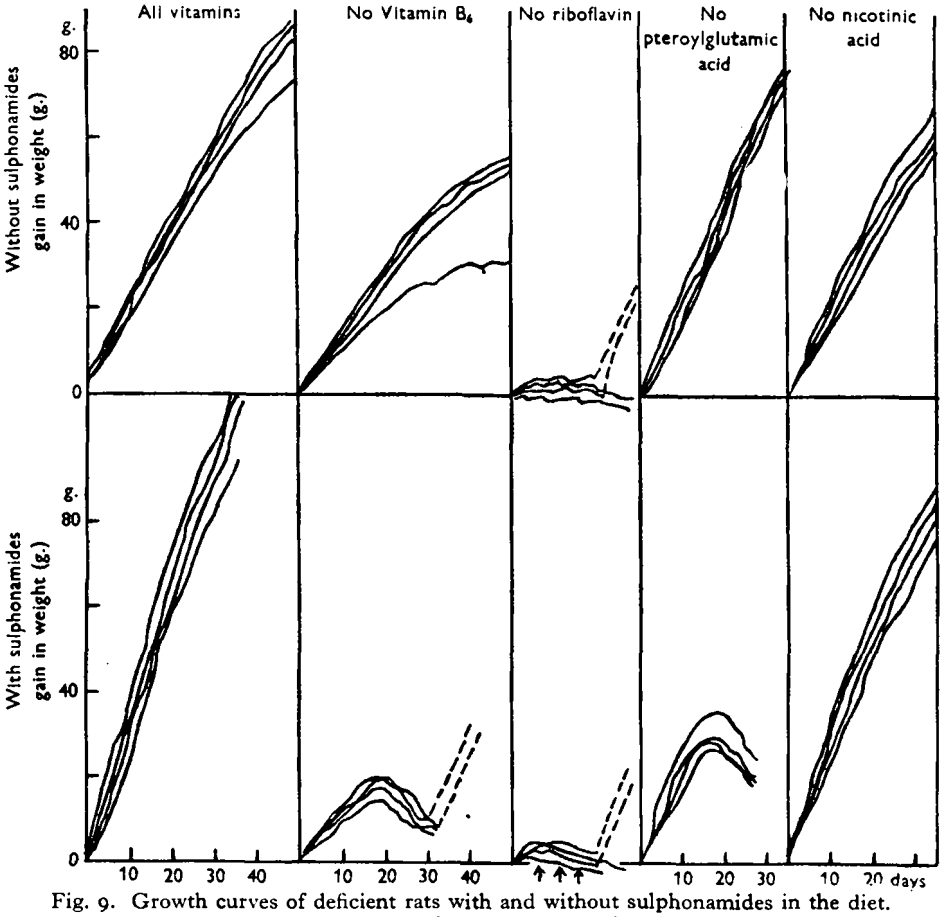


Fig. 9. Growth curves of deficient rats with and without sulphonamides in the diet. (↑ Doses of riboflavin, 70 μg.)

Recently Kauer, DuVall & Alquist (1947) synthesized the ε-isomer and Chaix (1948) reported experiments on bacteria, moulds, yeasts and protozoa with the α-, β-, γ-, δ- and ε-isomers of hexachloro*cyclo*hexane. Some micro-organisms were sensitive to the δ-isomer, others to the γ-isomer. She concluded that the antivitamin theory of Kirkwood & Phillips (1946) cannot explain all her findings.

Choline

The triethyl analogue of choline prevents the kidney haemorrhage occurring in choline deficiency (Welch, 1941); it was earlier found lypotropic (Channon & Smith, 1936). It did not, however, prevent the occurrence of paralysis, and did not support

the growth of fowls, in choline deficiency (Jukes, 1941; Moyer & du Vigneaud, 1942). On the other hand, Keston & Wortis (1946) found that it acted as a choline antagonist in mice, and that its effect could be reversed by equal amounts of choline. In isolated frog-muscle preparations it blocked the action of choline but not that of acetycholine. From these rather divergent findings it is difficult to decide how far the definition of an antivitamin (see p. 375) applies to this substance. In any case, the biochemical role of choline seems to be very different from that of other B-vitamins.

Zoopherin

Zoopherin, a new vitamin present in liver, has been described by Zucker, Zucker, Babcock & Hollister (1948). It may be identical with the cow-manure factor (Rubin & Bird, 1946) and possibly with the Animal Protein Factor of Cary, Hartman, Dryden & Likely (1946) and with vitamin B_{12} (Shorb, 1948). No effective antivitamins have yet been discovered for this nutrient, but an increased need for zoopherin or similar liver factors was observed when experimental animals were given thyroid (Betheil, Wiebelhaus & Lardy, 1947; Ershoff, 1947).

General conclusions

Only a few of the toxamins described above occur in nature, but these few have proved important in the causation of deficiency diseases. The majority of these toxic factors has been tested experimentally and this gives a sound foundation for future research. The structural analogues have proved useful for elucidating the active groups in a vitamin molecule and for studying severe deficiencies brought about by means other than simple primary deficiences. But, for a proper understanding of the biochemical action of antivitamins, it will be necessary to study the actual chemical changes occurring in the vitamin molecule during its action. Why, for instance, are there in the aneurin molecule various specific groups necessary for an efficient decarboxylation of pyruvic acid? Once the thermodynamics of these changes are understood, better and more efficient antivitamins may be devised which may prove of theoretical and practical importance.

REFERENCES

Abderhalden, E. (1947). *Z. Vitamin-, Hormon-, Fermentforsch.* **1**, 55.
Ågren, G. (1946). *Acta Physiol. Scand.* **11**, 344.
Albert, P. W., Scheer, B. T. & Deuel, H. J. Jr. (1948). *J. biol. Chem.* **175**, 479.
Auhagen, E. (1942). *Hoppe-Seyl. Z.* **274**, 48.
Bauer, D. J. (1948). *Nature, Lond.*, **161**, 852.
Beadle, G. W., Mitchell, H. K. & Nyc, J. F. (1947). *Proc. nat. Acad. Sci., Wash.*, **33**, 155.
Beerstecher, E. Jr. & Shive, W. (1946). *J. biol. Chem.* **164**, 53.
Beiler, J. M. & Martin, G. J. (1947). *J. biol. Chem.* **169**, 345.
Bergel, F. & Todd, A. R. (1937). *J. chem. Soc.* p. 1504.
Berger, J. & Avery, G. S. Jr. (1944). *Amer. J. Bot.* **31**, 199.
Betheil, J. J., Wiebelhaus, V. D. & Lardy, H. A. (1947). *J. Nutrit.* **34**, 431.
Bhagvat, K. & Devi, P. (1944). *Indian J. med. Res.* **32**, 123.
Bonner, D. (1948). *Proc. nat. Acad. Sci., Wash.*, **34**, 5.
Borrow, A., Fowden, L., Stedman, M. M., Waterlow, J. C. & Webb, R. A. (1948). *Lancet*, **254**, 752.
Buchman, E. R., Heegaard, E. & Bonner, J. (1940). *Proc. nat. Acad. Sci., Wash.*, **26**, 561.
Buchman, E. R. & Richardson, E. M. (1945). *J. Amer. chem. Soc.* **67**, 395.
Buston, H. W., Jacobs, S. E. & Goldstein, A. (1946). *Nature, Lond.*, **158**, 22.
Carpenter, K. J., Harris, L. J. & Kodicek, E. (1948–9). *Brit. J. Nutrit.* **2**, vii.

Carpenter, K. J. & Kodicek, E. (1948–9). *Brit. J. Nutrit.* **2**, ix.
Cary, C. A., Hartman, A. M., Dryden, L. P. & Likely, G. D. (1946). *Fed. Proc.* **5**, 128.
Cerecedo, L. R. & Foy, J. R. (1944). *Arch. Biochem.* **5**, 207.
Chaix, P. (1948). *Trans. Int. Congr. Antivitamins, Lyon.* (In the Press.)
Channon, H. J. & Smith, J. A. B. (1936). *Biochem. J.* **30**, 115.
Cosulich, D. B. & Smith, J. M. Jr. (1948). *J. Amer. chem. Soc.* **70**, 1922.
Daniel, L. J. & Norris, L. C. (1947). *J. biol. Chem.* **170**, 747.
Daniel, L. J., Norris, L. C., Scott, M. L. & Heuser, G. F. (1947). *J. biol. Chem.* **169**, 689.
Deutsch, H. F. & Hasler, A. D. (1943). *Proc. Soc. exp. Biol., N.Y.*, **53**, 63.
Dittmer, K., Melville, D. B. & Vigneaud, V. du (1944). *Science*, **99**, 203.
Dittmer, K. & Vigneaud, V. du (1944). *Science*, **100**, 129.
Dodds, E. C. (1948). *Trans. 8th Congr. biol. Chem. Paris.* (In the Press.)
Dubos, R. J. (1946). *Proc. Soc. exp. Biol., N.Y.*, **63**, 317.
Duschinsky, R. & Rubin, S. H. (1948). *J. Amer. chem. Soc.* **70**, 2546.
Eakin, R. E., Snell, E. E. & Williams, R. J. (1941). *J. biol. Chem.* **140**, 535.
Editorial (1948). *Blood*, **3**, 1059.
Elvehjem, C. A. (1948). *Fed. Proc.* **7**, 410.
Emerson, G. A. (1947). *Fed. Proc.* **6**, 406.
Emerson, G. A. & Southwick, P. (1945). *J. biol. Chem.* **160**, 169.
Emerson, G. A. & Tishler, M. (1944). *Proc. Soc. exp. Biol., N.Y.*, **55**, 184.
Emerson, G. A., Wurtz, E. & Johnson, O. H. (1945). *J. biol. Chem.* **160**, 165.
English, J. P., Clapp, R. C., Cole, Q. P., Halverstadt, I. F., Lampen, J. O. & Roblin, R. O. Jr. (1945). *J. Amer. chem. Soc.* **67**, 295.
Erlenmeyer, H. (1948). *Trans. Int. Congr. Antivitamins, Lyon.* (In the Press.)
Erlenmeyer, H., Bloch, H. & Kiefer, H. (1942). *Helv. chim. Acta*, **25**, 1066.
Erlenmeyer, H., Waldi, D. & Sorkin, E. (1948). *Helv. chim. Acta*, **31**, 32.
Erlenmeyer, H. & Würgler, W. (1942). *Helv. chim. Acta*, **25**, 249.
Ershoff, B. H. (1947). *Proc. Soc. exp. Biol., N.Y.*, **64**, 500.
Ershoff, B. H. (1948). *Physiol. Rev.* **28**, 107.
Euler, H. v. & Karrer, P. (1946). *Helv. chim. Acta*, **29**, 353.
Evans, C. A., Carlson, W. E. & Green, R. G. (1942). *Amer. J. Path.* **18**, 79.
Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F. Jr. & Wolff, J. A. (1948). *New Engl. J. Med.* **238**, 787.
Fildes, P. (1941). *Brit. J. exp. Path.* **22**, 293.
Foster, J. W. (1944). *J. Bact.* **48**, 97.
Franklin, A. L., Stokstad, E. L. R., Belt, M. & Jukes, T. H. (1947). *J. biol. Chem.* **169**, 427.
Franklin, A. L., Stokstad, E. L. R. & Jukes, T. H. (1947). *Proc. Soc. exp. Biol., N.Y.*, **65**, 368.
Franklin, A. L., Stokstad, E. L. R. & Jukes, T. H. (1948). *Proc. Soc. exp. Biol., N.Y.*, **67**, 398.
Green, R. G., Carlson, W. E. & Evans, C. A. (1941). *J. Nutrit.* **21**, 243.
Haag, J. R. & Weswig, P. H. (1948). *Fed. Proc.* **7**, 157.
Haag, J. R., Weswig, P. H. & Freed, A. M. (1947). *Fed. Proc.* **6**, 408.
Haagen-Smit, A. J., Leech, W. D. & Bergner, W. R. (1942). *Amer. J. Bot.* **29**, 500.
Hankes, L. V., Henderson, L. M., Brickson, W. L. & Elvehjem, C. A. (1948). *J. biol. Chem.* **174**, 873.
Hart, E. B., McCollum, E. V. & Steenbock, H. (1911). *Res. Bull. Wis. agric. Exp. Sta.* no. 17.
Hart, E. B., Miller, W. S. & McCollum, E. V. (1916). *J. biol. Chem.* **25**, 239.
Heidelberger, C., Gullberg, M. E., Morgan, A. F. & Lepkovsky, S. (1948). *J. biol. Chem.* **175**, 471.
Henderson, L. M., Deodhar, T., Krehl, W. A. & Elvehjem, C. A. (1947). *J. biol. Chem.* **170**, 261.
Hertz, R. (1946). *Physiol. Rev.* **26**, 479.
Heyl, D., Luz, E., Harris, S. A. & Folkers, K. (1948). *J. Amer. chem. Soc.* **70**, 1670.
Hitchings, G. H., Elion, G. B. & VanderWerff, H. (1948). *J. biol. Chem.* **174**, 1037.
Hutchings, B. L., Mowat, J. H., Oleson, J. J., Stokstad, E. L. R., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J. & Subbarow, Y. (1947). *J. biol. Chem.* **170**, 323.
Jacobsohn, K. P. & Azevedo, M. D. (1947). *Arch. Biochem.* **14**, 83.
Jukes, T. H. (1941). *J. Nutrit.* **21**, suppl. p. 13.
Kalckar, H. M., Kjeldgaard, N. O. & Klenow, H. (1948). *J. biol. Chem.* **174**, 771.
Kaplan, I. I. (1944). *Amer. J. med. Sci.* **207**, 733.
Kauer, K. C., DuVall, R. B. & Alquist, F. N. (1947). *Industr. Engng Chem.* (Industr. ed.), **39**, 1335.
Keston, A. S. & Wortis, S. B. (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 439.
Kingsley, H. N. & Parson, H. T. (1947). *J. Nutrit.* **34**, 321.
Kirkwood, S. & Phillips, P. H. (1946). *J. biol. Chem.* **163**, 251.
Knight, B. C. J. G. (1945). *Vitamins and Hormones*, **3**, 108.
Knight, B. C. J. G. (1946). *Proc. Nutr. Soc.* **4**, 116.
Kodicek, E., Carpenter, K. J. & Harris, L. J. (1946). *Lancet*, **251**, 491.

Kodicek, E., Carpenter, K. J. & Harris, L. J. (1947). *Lancet*, **253**, 616.
Krampitz, L. O. & Woolley, D. W. (1944). *J. biol. Chem.* **152**, 9.
Krehl, W. A., Carvalko, A. & Cowgill, G. R. (1947). *Fed. Proc.* **6**, 413.
Krehl, W. A., Henderson, L. M., Huerga, J. de la & Elvehjem, C. A. (1946). *J. biol. Chem.* **166**, 531.
Krehl, W. A., Sarma, P. S., Teply, L. J. & Elvehjem, C. A. (1946). *J. Nutrit.* **31**, 85.
Krehl, W. A., Teply, L. J. & Elvehjem, C. A. (1945). *Science*, **101**, 283.
Kuhn, R. & Beinert, H. (1947). *Ber. dtsch. chem. Ges.* **80**, 101.
Kuhn, R., Weygand, F. & Möller, E. F. (1943). *Ber. dtsch. chem. Ges.* **76**, 1044.
Lampen, J. O. & Jones, M. J. (1946). *J. biol. Chem.* **166**, 435.
Lampen, J. O. & Jones, M. J. (1947). *J. biol. Chem.* **170**, 133.
Lilley, V. G. & Leonian, L. H. (1944). *Science*, **99**, 205.
Lipmann, F., Kaplan, N. O., Novelli, G. D., Tuttle, L. C. & Guirard, B. M. (1947). *J. biol. Chem.* **167**, 869.
Little, P. A., Sampath, A., Paganelli, V., Locke, E. & Subbarow, Y. (1948). *Trans. N.Y. Acad. Sci.* **10**, 91.
Lutz, A. (1948). *C.R. Acad. Sci., Paris*, **226**, 281.
McCollum, E. V., Simmonds, N. & Pitz, W. (1916). *J. biol. Chem.* **25**, 105.
McIlwain, H. (1940*a*). *Brit. J. exp. Path.* **21**, 136.
McIlwain, H. (1940*b*). *Nature, Lond.*, **146**, 653.
McIlwain, H. & Hawking, F. (1943). *Lancet*, **244**, 449.
Martin, G. J., Avakian, S. & Moss, J. (1948). *J. biol. Chem.* **174**, 495.
Martin, G. J. & Beiler, J. M. (1947). *Arch. Biochem.* **15**, 201.
Martin, G. J., Tolman, L. & Moss, J. (1947). *Arch. Biochem.* **12**, 318.
Matti, J., Nitti, F., Morel, M. & Lwoff, A. (1941). *Ann. Inst. Pasteur*, **67**, 240.
Mellanby, E. (1926). *J. Physiol.* **61**, xxiv.
Mellanby, E. (1937). *Toxamins in Food.* In *Perspectives in Biochemistry*, p. 318. [J. Needham and D. E. Green, editors.] Cambridge: University Press.
Melnick, D., Hochberg, M. & Oser, B. L. (1945). *J. Nutrit.* **30**, 81.
Meyer, L. M. (1948). *Trans. N.Y. Acad. Sci.* **10**, 99.
Mitchell, H. K. & Nyc, J. F. (1948). *Proc. nat. Acad. Sci., Wash.*, **34**, 1.
Mitchell, H. K., Nyc, J. F. & Owen, R. D. (1948). *J. biol. Chem.* **175**, 433.
Möller, E. F. & Birkofer, L. (1942). *Ber. dtsch. chem. Ges.* **75**, 1108.
Möller, E. F., Zima, O., Jung, F. & Moll, T. (1939). *Naturwissenschaften*, **27**, 228.
Moore, P. H. (1914). *Rep. exp. Fms Can.*, p. 21.
Moyer, A. W. & Vigneaud, V. du (1942). *J. biol. Chem.* **143**, 373.
Mushett, G. W., Stebbins, R. B. & Barton, M. N. (1947). *Trans. N.Y. Acad. Sci.* **9**, 291.
Neilands, J. B. (1947). *J. Fish. Res. Bd Can.* **7**, 94.
Nelson, M. M. & Evans, H. M. (1947). *Proc. Soc. exp. Biol., N.Y.*, **66**, 299.
Nelson, M. M. & Evans, H. M. (1948). *Proc. Soc. exp. Biol., N.Y.*, **68**, 274.
Nyc, J. F. & Mitchell, H. K. (1948). *J. Amer. chem. Soc.* **70**, 1847.
Oleson, J. J., Hutchings, B. L. & Subbarow, Y. (1948). *J. biol. Chem.* **175**, 359.
Oppel, T. W. (1948). *Amer. J. med. Sci.* **215**, 76.
Ott, W. H. (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 125.
Perlman, D. (1946). *J. Bact.* **52**, 501.
Pfaltz, H. (1943). *Z. Vitaminforsch.* **13**, 236.
Plattner, P. A. & Clausen-Kaas, N. (1945*a*). *Helv. chim. Acta*, **28**, 188.
Plattner, P. A. & Clausen-Kaas, N. (1945*b*). *Experientia*, **1**, 195.
Pollack, M. A. (1943). *J. Amer. chem. Soc.* **65**, 1335.
Porter, C. C., Clark, I. & Silber, R. H. (1947). *J. biol. Chem.* **167**, 573.
Price, E. L., Marquette, M. M. & Parsons, H. T. (1947). *J. Nutrit.* **34**, 311.
Putney, W. W. (1945). *J. Amer. vet. med. Ass.* **106**, 164.
Radeleff, R. D. (1945). *Vet. Med.* **40**, 280.
Rhoads, C. P. & Abels, J. C. (1943). *J. Amer. med. Ass.* **121**, 1261.
Robbins, W. J. (1941). *Proc. nat. Acad. Sci., Wash.*, **27**, 419.
Roblin, R. O. Jr. (1946). *Chem. Rev.* **38**, 255.
Rogers, L. L. & Shive, W. (1947). *J. biol. Chem.* **169**, 57.
Rosen, F. & Perlzweig, W. A. (1947). *Arch. Biochem.* **15**, 111.
Rubin, M. & Bird, H. R. (1946). *J. biol. Chem.* **163**, 387.
Rubin, S. H., Cooperman, J. M., Moore, M. E. & Scheiner, J. (1948). *J. Nutrit.* **35**, 499.
Rydon, H. N. (1948). *Trans. Int. Congr. Antivitamins, Lyon.* (In the Press.)
Sarett, H. P. (1946). *J. biol. Chem.* **162**, 87.
Sarett, H. P. & Cheldelin, V. H. (1944). *J. biol. Chem.* **156**, 91.
Schopfer, W. H. (1948). *Int. Rev. Vit. Res.* **20**, 116.
Schultz, F. (1940). *Hoppe-Seyl. Z.* **256**, 113.

Sealock, R. R. & Goodland, R. L. (1944). *J. Amer. chem. Soc.* **66**, 507.
Sealock, R. R. & Livermore, A. H. (1944). *J. biol. Chem.* **156**, 379.
Seeger, D. R., Smith, J. M. Jr. & Hultquist, M. E. (1947). *J. Amer. chem. Soc.* **69**, 2567.
Sevag, M. G., Shelburne, M. & Mudd, S. (1942). *J. gen. Physiol.* **25**, 805.
Shive, W., Ackerman, W. W., Ravel, J. M. & Sutherland, J. E. (1947). *J. Amer. chem. Soc.* **69**, 2576.
Shive, W., Eakin, R. E., Harding, W. M., Ravel, J. M. & Sutherland, J. E. (1948). *J. Amer. chem. Soc.* **70**, 2299.
Shive, W. & Macow, J. (1946). *J. biol. Chem.* **162**, 451.
Shive, W. & Roberts, E. C. (1946). *J. biol. Chem.* **162**, 463.
Shorb, M. S. (1948). *Science*, **107**, 397.
Smith, D. C. & Proutt, L. M. (1944). *Proc. Soc. exp. Biol., N.Y.*, **56**, 1.
Smith, J. M. Jr., Cosulich, D. B., Hultquist, M. E. & Seeger, D. R. (1948). *Trans. N.Y. Acad. Sci.* **10**, 82.
Snell, E. E. (1941). *J. biol. Chem.* **139**, 975.
Snell, E. E., Chan, L., Spiridanoff, S., Way, E. L. & Leake, C. D. (1943). *Science*, **97**, 168.
Snell, E. E., Kitay, E. & McNutt, W. S. (1948). *J. biol. Chem.* **175**, 473.
Soodak, M. & Cerecedo, L. R. (1944). *J. Amer. chem. Soc.* **66**, 1988.
Sprince, H. & Woolley, D. W. (1944). *J. exp. Med.* **80**, 213.
Sprince, H. & Woolley, D. W. (1945). *J. Amer. chem. Soc.* **67**, 1734.
Stoerk, H. C. & Pappenheimer, A. M. (1948). *Fed. Proc.* **7**, 281.
Stokes, J. L. (1944). *J. Bact.* **48**, 201.
Stokstad, E. L. R., Regan, M., Franklin, A. L. & Jukes, T. H. (1948). *Fed. Proc.* **7**, 193.
Swendseid, M. E., Wittle, E. L., Moersch, G. W., Bird, O. D. & Brown, R. (1948). *Fed. Proc.* **7**, 299.
Sydenstricker, V. P., Singal, S. A., Briggs, A. P., DeVaughn, N. M. & Isbell, H. (1942). *J. Amer. med. Ass.* **118**, 1199.
Tatum, E. L. & Bell, T. T. (1946). *Amer. J. Bot.* **33**, 15.
Unna, K. (1943). *Proc. Soc. exp. Biol., N.Y.*, **54**, 55.
Wallerstein, J. S. & Stern, K. G. (1945). *J. biol. Chem.* **158**, 1.
Welch, A. D. (1941). *J. biol. Chem.* **137**, 173.
Welch, A. D. (1945). *Physiol. Rev.* **25**, 687.
Welch, A. D., Heinle, R. W., Sharpe, G., George, W. L. & Epstein, M. (1947). *Proc. Soc. exp. Biol., N.Y.*, **65**, 364.
Weswig, P. H., Freed, A.M. & Haag, J. R. (1946). *J. biol. Chem.* **165**, 737.
Williams, R. D. (1944). *J. biol. Chem.* **156**, 85.
Williams, R. R. (1927). *Biochem. J.* **21**, 1349.
Winterbottom, R., Clapp, J. W., Miller, W. H., English, J. P. & Roblin, R. O. Jr. (1947). *J. Amer. chem. Soc.* **69**, 1393.
Woll, E. (1948). *Trans. N.Y. Acad. Sci.* **10**, 83.
Woods, D. D. (1940). *Brit. J. exp. Path.* **21**, 74.
Woods, D. D. (1948). *Trans. Int. Congr. Antivitamins, Lyon.* (In the Press.)
Woods, D. D. & Fildes, P. (1940). *J. Soc. chem. Ind., Lond.*, **59**, 133.
Woolley, D. W. (1941*a*). *J. biol. Chem.* **141**, 997.
Woolley, D. W. (1941*b*). *J. exp. Med.* **73**, 487.
Woolley, D. W. (1944). *J. biol. Chem.* **154**, 31.
Woolley, D. W. (1945). *J. biol. Chem.* **157**, 455.
Woolley, D. W. (1946*a*). *Advan. Enzymol.* **6**, 129.
Woolley, D. W. (1946*b*). In *Currents in Biochemical Research*, p. 357. [D. E. Green, editor.] New York: Interscience Publishers, Inc.
Woolley, D. W. (1946*c*). *J. biol. Chem.* **162**, 179.
Woolley, D. W. (1946*d*). *J. biol. Chem.* **163**, 773.
Woolley, D. W. (1946*e*). *J. biol. Chem.* **166**, 783.
Woolley, D. W. (1947). *Physiol. Rev.* **27**, 308.
Woolley, D. W. (1948*a*). *Trans. Int. Congr. Antivitamins, Lyon.* (In the Press.)
Woolley, D. W. (1948*b*). *J. biol. Chem.* **176**, 1291.
Woolley, D. W. (1948*c*). Private communication.
Woolley, D. W. & Collyer, M. L. (1945). *J. biol. Chem.* **159**, 263.
Woolley, D. W. & Longsworth, L. G. (1942). *J. biol. Chem.* **142**, 285.
Woolley, D. W. & Pringle, A. (1948). *J. biol. Chem.* **174**, 327.
Woolley, D. W., Strong, F. M., Madden, R. J. & Elvehjem, C. A. (1938). *J. biol. Chem.* **124**, 715.
Woolley, D. W. & White, A. G. C. (1943*a*). *J. biol. Chem.* **149**, 285.
Woolley, D. W. & White, A. G. C. (1943*b*). *J. exp. Med.* **78**, 489.
Woolley, D. W. & White, A. G. C. (1943*c*). *Proc. Soc. exp. Biol., N.Y.*, **52**, 106.
Wyss, O. (1943). *J. Bact.* **46**, 483.
Yudkin, W. H. (1945). *Proc. Soc. exp. Biol., N.Y.*, **60**, 268.
Zucker, L. M., Zucker, T. F., Babcock, V. & Hollister, P. (1948). *Arch. Biochem.* **16**, 115.