Summary

Clinical and experimental work suggests that nutritional macrocytic anaemia is not due to a lack of Castle's extrinsic factor, if by this is meant a substance that reacts with the intrinsic factor to form the liver principle. In uncomplicated cases in men and monkeys, which have received no previous treatment with fractions of the vitamin B_2 complex, highly purified liver extracts are inactive. The deficiency causing the anaemia is in some component of the vitamin B_2 complex, at present unidentified. This factor is active when given parenterally and does not, therefore, need the presence of normal gastric juice for its proper functioning. It is suggested that the condition is due to a failure of the liver principle to promote the maturation of the erythroblasts owing to a failure in cellular metabolism due to the absence of one or other members of the vitamin B_2 complex which act as essential components of cellular enzyme systems.

REFERENCES

Ahmad, N. (1944). *Indian med. Gaz.* **72**, 414.
Campbell, C. J., Brown, R. A. and Emmett, A. D. (1944). *J. biol. Chem.* **152**, 483.
Cartwright, G. E., Wintrobe, M. M. and Humphreys, S. (1944). *J. biol. Chem.* **153**, 171.
Castle, W. B., Ross, J. B., Davidson, C. S., Burchenal, J. H., Fox, K. J. and Ham, T. H. (1944). *Science*, **100**, 81.
Foy, H. and Kondi, A. (1939). *Lancet*, **237**, 360.
Fuchs, H. and Wisselwick, A. (1939). *Klin. Wschr.* **18**, 722.
Handler, P. and Featherston, W. P. (1943). *J. biol. Chem.* **151**, 394.
Hogan, A. G. and Parrott, E. M. (1940). *J. biol. Chem.* **132**, 507.
Miller, D. K. and Rhoads, C. P. (1933). *J. exp. Med.* **58**, 585.
Moore, C. V., Vilter, R., Minnich, V. and Spies, T. D. (1944). *J. Lab. clin. Med.* **29**, 1227.
Napier, L. E. (1939). *Indian med. Gaz.* **74**, 1.
Robinson, F. A. (1944). *Nature, Lond.*, **153**, 478.
Spector, H., Maas, A. R., Michael, L., Elvehjem, C. A. and Hart, E. B. (1943). *J. biol. Chem.* **150**, 75.
Stannus, H. S. (1944). *Brit. med. J.* ii, 103.
Waisman, H. A. (1944). *Proc. Soc. exp. Biol., N.Y.*, **55**, 69.
Wills, L. (1934). *Indian J. med. Res.* **21**, 669.
Wills, L., Clutterbuck, P. W. and Evans, B. D. F. (1939). *Biochem. J.* **31**, 2136.
Wills, L. and Evans, B. D. F. (1938). *Lancet*, **235**, 416.

Discussion

Dr. F. Prescott (The Wellcome Research Institution, 183–193 Euston Road, London, N.W.1) opener: In connexion with the biosynthesis of the B vitamins in the human gut, to which Dr. Platt referred in his paper, there is a question which until recently has been overlooked. If it is assumed that the intestinal flora of man can synthesize vitamin B_1, riboflavin and nicotinic acid, are these vitamins available to the host? A recent paper suggests that they are not. Alexander and Landwehr (1945) of Harvard have shown that the vitamin B_1 in human faeces is largely within the bodies of the bacteria and not free in the lumen of the gut. It may, therefore, not be available to the host. Furthermore, most of the vitamin B_1 is present as co-carboxylase, which cannot be absorbed as such and must first be split by dephosphorylating enzymes. It is doubtful if these are present in the large intestine. Najjar and Holt (1943) in their paper on the biosynthesis of vitamin B_1 in man assumed that the vitamin synthesized by the intestinal bacteria was available to the host

because, if vitamin B_1 was given in an enema, some of it was absorbed from the large gut, but the quantity, 50 mg., used in this experiment was unphysiological. When Alexander and Landwehr used physiological amounts of less than 2 mg. in the enema, they found no evidence of the absorption of vitamin B_1 or co-carboxylase. The subject obviously requires further investigation. If it can be shown that man can utilize the B vitamins synthesized by his intestinal flora, as after all the rodents and the herbivores can, our ideas on vitamin requirements will need revision.

There is evidence that the degree of biosynthesis that occurs varies considerably from person to person, even on the same diet. This might explain the divergent views held on the human requirements of the B vitamins. Keys, Henschel, Mickelsen and Brozek (1943), for example, kept volunteers on diets that provided only 0·23 mg. of vitamin B_1 per 1000 Calories as against 0·6 mg. per 1000 Calories recommended by the U.S.A. National Research Council (1941), yet the subjects got along quite well on this low intake. The same workers (Keys, Henschel, Mickelsen, Brozek and Crawford, 1944) were unable to find evidence of riboflavin deficiency in volunteers kept on diets containing 0·9 mg. riboflavin daily, although others have reported deficiency symptoms on an intake of over 1 mg. daily.

It is well known that a diet containing much maize is likely to cause pellagra in man and blacktongue in dogs, yet the addition of liberal amounts of milk to such a diet can prevent pellagra, although the nicotinic acid content of 0·5 mg. per 100 ml. milk is negligible. Do maize and milk, respectively, inhibit and promote the growth or activity of the intestinal organisms which synthesize nicotinic acid?

It has been observed that patients receiving massive doses of sulphaguanidine, one of the less soluble sulphonamides, show mental symptoms similar to those seen in pellagrins, confusional psychosis, lack of concentration, and disorientation (Crofton and Diggle, 1944). It has been argued that the sulphaguanidine inhibits the biosynthesis of nicotinic acid and that the patients therefore suffer from the mental symptoms of an acute nicotinic acid deficiency. A far more likely explanation is that the respiratory enzyme system, of which the phosphopyridine nucleotides are components, is disturbed. This system is concerned with the metabolism of carbohydrate, which is used exclusively by nervous tissue as a source of energy. These mental symptoms may, therefore, be produced by cerebral anoxia, the term anoxia being used in its widest physiological sense. Irradiation sickness, due to exposure to X-rays and radium, is manifested by nervous and gastro-intestinal symptoms and by changes in the blood, such as anaemia and leucopenia. May not the nervous symptoms and the blood dyscrasia be due to a similar mechanism, namely, interference with the respiratory enzyme systems, of which vitamin B_1, nicotinic acid and riboflavin are components? There are reports on the prevention and relief of irradiation sickness with liver extracts and nicotinic acid.

Last year Howat (1944) recorded that the onset of steatorrhoea, a vitamin B deficiency syndrome, often occurred *after* sulphaguanidine therapy. Was this due to the bacteriostatic effect of the sulphaguanidine on the flora synthesizing B vitamins in the intestine? This

synthesizing activity of the intestinal flora may not be recovered for some time after the sulphaguanidine is withdrawn and after the intestinal bacterial counts have returned to normal (Gant, Ransone, McCoy and Elvehjem, 1943).

In her paper on the vitamin B complex and anaemia, Dr. Wills mentions that she has shown that the haemopoietic principle of liver and marmite is not vitamin B_1, riboflavin, nicotinic acid, pyridoxin or pantothenic acid. Castle, Ross, Davidson, Burchenal, Fox and Ham (1944) have recently enlarged this list to include *p*-aminobenzoic acid, choline, inositol, biotin, folic acid and xanthopterin, none of which gives a haemopoietic response in pernicious anaemia patients. So far, then, none of the isolated factors of the vitamin B complex has been shown to have any haemopoietic properties in man. In spite of this, vitamin B_1, riboflavin, nicotinic acid, pantothenic acid and vitamin B_6 are often added by manufacturers to liver extract. In this country liver extracts given by injection serve only one purpose, the cure of pernicious anaemia and most macrocytic anaemias. There is no clinical evidence that the haemopoietic activity of liver is enhanced by any of these added vitamins. A preliminary report was published last year (Sharp, Heide and Wolter, 1944) stating that vitamin B_c, the chick anti-anaemia factor, was of some value in the treatment of resistant cases of anaemia, but it has never been confirmed.

I should like to mention here the treatment of drug anaemias with vitamins of the B complex. The sulphones, promin and promizole, which are under trial for the treatment of tuberculosis, produce an anaemia, stated to be both preventible and curable by administration of vitamin B_c (Higgins, 1944). The anaemia produced in animals by poorly absorbed sulphonamides, such as succinylsulphathiazole, is said to be cured also by folic acid (Saslaw, Wilson, Doan and Schwab, 1943). The haemolytic anaemia that occurs in animals given phenothiazine, an anthelmintic, is intensified by lack of the vitamin B complex (Collier and Mack, 1944).

It is possible that many of the clinical conditions discussed at this meeting, nutritional anaemia, drug anaemia, the blood dyscrasias produced by sulphones and sulphonamides, the mental manifestations of sulphonamide toxicity, and irradiation sickness, all have a common aetiological factor, namely deficiency of essential factors of the vitamin B complex or inhibition of their enzyme systems, resulting in anoxia of the haemopoietic and nervous systems.

REFERENCES

Alexander, B. and Landwehr, G. (1945). *Science*, **101**, 229.
Castle, W. B., Ross, J. B., Davidson, C. S., Burchenal, J. H., Fox, H. J. and Ham, T. H. (1944). *Science*, **100**, 81.
Collier, H. B. and Mack, G. E. (1944). *Canad. J. Res.* **22**, 1.
Crofton, J. W. and Diggle, G. (1944). *Brit. med. J.* i, 367.
Gant, O. K., Ransone, B., McCoy, E. and Elvehjem, C. A. (1943). *Proc. Soc. exp. Biol., N.Y.*, **52**, 276.
Higgins, G. M. (1944). *Proc. Mayo Clin.* **19**, 329.
Howat, H. T. (1944). *Lancet*, **247**, 560.
Keys, A., Henschel, A. F., Mickelsen, O. and Brozek, J. M. (1943). *J. Nutrit.* **26**, 399.
Keys, A., Henschel, A. F., Mickelsen, O., Brozek, J. M. and Crawford, J. H. (1944). *J. Nutrit.* **27**, 165.

Najjar, V. A., and Holt, L. E. (Jr.) (1943). *J. Amer. med. Ass.* **123**, 683.
Saslaw, S., Wilson, H. E., Doan, C. A. and Schwab, J. L. (1943). *Science*, **97**, 514.
Sharp, E. A., Heide, E. C. V. and Wolter, J. G. (1944). *J. Amer. med. Ass.* **124**, 734.
U.S.A. National Research Council. Committee on Food and Nutrition (1941). *J. Amer. med. Ass.* **116**, 2601.

Wing Commander T. F. Macrae, R.A.F.V.R. (R.A.F. Institute of Pathology and Tropical Medicine): The experiments on the nature of the factor responsible for the nutritional anaemia in monkeys, in which we were collaborating with Dr. Wills, were abruptly stopped by the beginning of the war. We had reached an interesting stage and thought that we were making some progress but the animals had to be killed. It is impossible to say which factor was involved, but aneurin, nicotinic acid, pyridoxin, riboflavin and pantothenic acid were definitely excluded. The factor appeared to be one which was unknown in 1939 and may have been identical with one of two unidentified factors which we recognized at the time as essential for the growth of the rat.

Dr. E. Kodicek (Dunn Nutritional Laboratory, Cambridge): A recent publication by Watson and Castle (1945) gives further support to Dr. Wills' view that the factor or factors curative in nutritional macrocytic anaemia is different from Castle's extrinsic factor. In this connexion I should like to ask Dr. Wills whether she has seen any results from dosing with ascorbic acid. I observed an astonishing response to intravenous injections of ascorbic acid in one patient suffering from typical pernicious anaemia (Kodicek, 1934). The red cell count improved within 4 weeks from 1,840,000 to 4,500,000 and the patient had no relapse for 5 years during which she took ascorbic acid without any liver treatment. Other patients, however, did not respond to the same therapy. I mention these observations to underline the complexity of the problem.

REFERENCES

Kodicek, E. (1934). *Čas. čsl. Lék.* **73**, 501.
Watson, J. and Castle, W. B. (1945). *Proc. Soc. exp. Biol., N.Y.*, **58**, 84.

Dr. C. F. Brockington (Public Health Department, Shire Hall, Warwick): I should like to ask Dr. Platt what is the explanation for the failure of the vitamins of the B group produced by fermentation to prevent the alcoholic diseases. Many of the alcoholic diseases are now known to be due to deficiencies of the vitamin B group, for example, neuritis to aneurin deficiency, the encephalopathies possibly to nicotinic acid deficiency, and cirrhosis to methionine or choline deficiency. If fermentation increases the potency of alcoholic drinks to the extent shown by Dr. Platt one would naturally assume that they would themselves prevent the onset of the deficiency diseases. Is the explanation that the amounts of the vitamin B group present in ordinary alcoholic drinks is quite insufficient or that absorption is inhibited? Possibly the alcoholic drinks referred to by Dr. Platt among African communities are of a higher potency.

Mr. R. L. Edwards (Putney): Dr. Wills said that dried yeast was ineffective in curing nutritional macrocytic anaemia which was curable

by crude yeast extracts. What kind of dried yeast was given, and what kind of yeast was used in making the yeast extracts?

Dr. R. L. M. Synge (Lister Institute, Chelsea Bridge Road, London, S.W.1): The name *riboflavin* is misleading in view of the presence in the molecule not of a ribose but of a ribitol residue, and should be altered since, even at this late date, it leads to misapprehensions and wrong formulation.

Dr. H. S. Stannus (13 Harley Street, London, W.1): Dr. Platt's reference to observations in central Africa has reminded me of notes I made in Africa nearly 40 years ago about native food habits. If we are to know anything about the nutrition of native races we must collect the most detailed information. Dietary surveys as usually carried out often fail to include much that is of great importance. Administrators know nothing about such matters, most medical men are little better informed, and students of nutrition visiting a native country would never hear of some important food habits. I remember that when I shot an antelope my native followers always squeezed the juice out of the green mass in the rumen and drank it; we now know that the juice contains many vitamins. They next took the small intestine and after washing it put lengths on a spit, grilled them lightly over a wood fire and ate them. One wonders whether the intestine contained an anti-anaemia factor.

At a certain season of the year clouds of a small fly which breeds in the Great Lakes flew ashore and coated the foliage of trees. The masses were shaken off into baskets, mixed with meal and baked into cakes. Fried locusts were much appreciated for their fat, while towards the north a particularly luscious caterpillar feeding on creepers on the lake foreshore was collected by the women for food; it also apparently was a good source of fat.

Mr. A. L. Bacharach (Glaxo Laboratories, Ltd., Greenford, Middlesex): Dr. Wills has pointed out that yeast extracts given by injection and effective against macrocytic anaemia cannot act by virtue of any extrinsic factor in Castle's sense, because they cannot enter into reaction with the gastric intrinsic factor. This presupposes that there is no re-excretion into any part of the alimentary tract where the intrinsic factor can act. Is there any experimental evidence on this point?

Dr. B. B. G. Nehaul (British Guiana): In British Guiana it is very difficult to get West Indian women to take marmite. Patients, who in hospital recover from anaemia, sooner or later return again for treatment. We find that it is not enough to treat patients and let them return to live under former conditions; it is not enough to cure them and give them food, it is necessary to educate them.

Dr. B. S. Platt: I should like to point out the need for caution in the interpretation of clinical conditions like those listed in Table 7 of my paper with Dr. Webb. They may not necessarily be signs of a dietary deficiency disease and, even when their nutritional origin is established, they need not be specifically related to insufficiency of any one food factor.

In reply *to Dr. Prescott:* I have not overlooked Alexander and Landwehr's (1945) criticism of the work of Najjar and Holt (1943), which

Dr. Prescott recounted in opening the discussion. It seems to me, however, doubtful whether, in view of the smallness of the dose used by the former authors and of the shortcomings of existing methods, their finding is at all conclusive.

Dr. Prescott's comment on the value of milk in preventing pellagra despite its low content of nicotinic acid reminds me of a communication made to the Biochemical Society (Platt and Glock, 1942), in which a condition called African scurvy was discussed. This could be cured by the addition of milk or meat to the African diet. It is my opinion that this African scurvy was probably superimposed on an insufficiency in the diet of vitamins of the B group, in particular of nicotinic acid or its amide.

To Dr. Brockington: Alcoholic drinks of the type of Kaffir beer contain, apart from alcohol, considerable amounts of all B vitamins. As such beverages become more sophisticated these vitamins are removed; in European beer, for example, yeast and other solid matter is filtered off, and only factors like riboflavin, present in solution, remain. Distilled liquors do not contain any of these factors.

REFERENCES

Alexander, B. and Landwehr, G. (1945). *Science*, **101**, 229.
Najjar, V. A. and Holt, L. E. (Jr.) (1943). *J. Amer. med. Ass.* **123**, 683.
Platt, B. S. and Glock, G. E. (1942). *Biochem. J.* **36**, xv.

Dr. L. Wills replied:

To Dr. Kodicek: I have only seen one case of macrocytic anaemia in which vitamin C was the limiting factor. The patient made a dramatic cure on large doses of the vitamin but relapsed later in spite of continued treatment.

To Mr. Edwards: All the autolysed yeast extracts were prepared from brewer's yeast. The extracts made from non-autolysed yeast were from either brewer's or distiller's yeast.

To Mr. Bacharach: I have considered the possibility of the excretion into the gut of fractions given parenterally but as such excretion does not occur in the stomach and probably not in the duodenum, two areas where the intrinsic factor is supposed to react with the extrinsic, I think this possibility highly improbable.

Chairman's Summing Up

Dr. L. J. Harris (Dunn Nutritional Laboratory, Cambridge): In trying to summarize some of the main impressions left by today's meeting, one's most outstanding impression, perhaps, is the extent and importance of the recent developments in our knowledge of the B_2 vitamins. Together, these substances now constitute an impressive, and not inconsiderable chapter in the joint sciences of biochemistry and nutrition.

Chemical Structures

It will be useful for summing up to return once again to the table shown at the beginning of the meeting (p. 82), with the object especially of noting the gaps in knowledge which still remain. Column 1 indicates whether