Vitamin B.

ALTHOUGH our knowledge of the constitution and nature of vitamin D is greater than our knowledge of the chemical composition of the other accessory factors, recent work suggests that it will not be long before it has advanced to a comparable stage. Progress has been particularly striking in the case of vitamin B, and has followed two lines: the physiological function of this compound in the animal economy has been investigated by a number of observers, whilst at the same time much information has been obtained as to its chemical nature and properties.

In this connexion the most important recent development is the confirmation of suggestive previous researches, which indicated that the substance which we call 'vitamin B,' consists of at least two compounds with different chemical properties and different physiological functions. In this point vitamin B is following the example set by vitamin A, and although at first sight this splitting of a compound of unknown chemical constitution into two similarly unknown bodies may seem to hamper further advances, yet the opposite is probably more nearly true, at any rate if we may take the differentiation of vitamin D from vitamin A, followed by the early recognition of the sterol nature of the former, as an example of what may be expected to occur in the future in the case of vitamin B.

Our present knowledge indicates that deficiency of vitamin B in the diet is associated with human beriberi and pellagra and avian polyneuritis: the latter disease is usually held to be the equivalent of human beriberi. The association of pellagra with deficiency of vitamin B has only recently been conclusively demonstrated by Goldberger and his co-workers. How a deficiency of the vitamin produces symptoms is not known, although many suggestions, based on experimental work, have been made. Recent investigations into its physiology have included both a direct attack on the problem and also an examination of its relationship to the three chief classes of foodstuffs, the proteins, fats, and carbohydrates, in the diet.

J. C. Drummond and G. F. Marrian (*Biochem. Jour.*, vol. 20, p. 1229; 1926), after a scrutiny of the literature, found that the only definite function attributed to vitamin B was that of stimulating tissue oxidations, based on a decrease in oxidative power of the body in conditions of vitamin deficiency. In a series of experiments the authors investigated this alleged function and definitely demonstrated that vitamin B bears no relationship to tissue oxidations. Thus muscles obtained from pigeons or rats suffering from vitamin B deficiency, decolorised methylene blue under anærobic conditions to the same extent as the equivalent muscles from normal birds or animals: and the oxygen consumption of pigeon breast muscle or liver was the same, whether the tissue had been obtained from normal birds or from those suffering from polyneuritis. The oxygen consumption of rats kept on a complete diet was compared with that of animals kept on a vitamin B deficient diet, and no difference between the two was detected until the rats in the latter group were almost moribund: for these experiments the animals were lightly anæsthetised with amytal to eliminate variations in the oxygen consumption due to movements and placed in a modified form of the closed-circuit type of apparatus for estimating the respiratory exchange.

Now the early symptoms of vitamin B deficiency in the animals used by Drummond and Marrian are loss of appetite, constipation, and lassitude: the last stage of lowered body temperature, laboured respirations, inco-ordination of the hind limbs and convulsions only lasts about twenty-four hours, and the fall in oxygen consumption is only observed when the body temperature has fallen below 33° C: if the animal is warmed up, the body temperature and the oxygen consumption return towards their usual level, with a temporary improvement in the animal's condition. These effects, however, are not peculiar to vitamin B deficiency itself, but occur in animals starved completely of food or kept undernourished on a complete diet, the fall in body temperature and oxygen consumption occurring as above as a terminal event.

The conditions of starvation and vitamin B deficiency also produce similar effects on the blood sugar, which is raised from 0·082 per cent. to 0·125 per cent. during the earlier stages of the experiment but falls to 0·02-0·03 per cent. with the fall in body temperature. From these experiments the authors conclude that the relationship between vitamin B and appetite is the most important factor in producing the results observed, and that oxidation processes are normal in vitamin B deficiency: the majority of the symptoms are due simply to starvation, following the loss of appetite. That the oxidation processes of the body are normal in vitamin B deficiency, except when starvation enters into the picture, has also been shown by B. A. Lavrov and S. M. Matsko (*Jour. de Biol. et de Méd. Experiment*, No. 9, p. 71; 1926).

Since pigeons have so often been used for experimental work on vitamin B, Drummond, working with S. K. Kon, has investigated the relationship of the symptoms of vitamin B deficiency to those of simple undernutrition in these birds also (*Biochem. Jour.*, vol. 21, p. 632; 1927). Each bird on the vitamin-deficient diet was controlled by another, which was only given to eat as much food as the former had consumed during the previous twenty-four hours, but was provided daily also with a liberal supply of vitamin B: in this way it was hoped to differentiate between the symptoms due to vitamin B deficiency and those due to simple undernutrition.

Of all the various symptoms observed by Drummond and Kon in these experiments, only the loss of appetite and the nervous symptoms appeared to be specifically caused by absence of vitamin B from the diet. Nervous symptoms appeared in 79 per cent. of the birds within a month, and in 47 per cent. a temporary spontaneous improvement was noted (Kon, *ibid.* p. 834): in these experiments the birds were kept on a synthetic diet of the type used in experiments with rats: on a polished rice diet, 60 per cent. developed symptoms, which were speedily followed by death, no remissions being observed. The failure of appetite is associated with delay in the emptying of the crop: if forcibly fed, the crop still fails to empty normally, and the major portion of the food is vomited, so that the birds obtain no more nourishment than if left to feed themselves. The authors offer as explanation of the temporary improvement of the symptoms in so many birds on a synthetic diet, the suggestion that the convulsions in some way liberate vitamin B from the tissues and so make it available to subserve its function in nutrition.

The cause of the nervous symptoms is obscure: the organic lesion can only be slight, since injection of a preparation containing the vitamin will restore a pigeon to a normal condition in a few hours. The characteristic head retraction in this bird bears some resemblance to the forced movements or attitudes which can be obtained by stimulation of the labyrinth or its central connexions, but the nervous symptoms occur in birds in which the labyrinth has been

destroyed (L. A. Tscherkes and T. M. Kuperman, *Jour. Biol. et Med. Exper.*, No. 8, p. 13; 1926). Nervous lesions demonstrable histologically have been described in both birds and rats. In the fowl, P. G. Culley (*Quart. Jour. Exp. Physiol.*, vol. 17, p. 65; 1927) has found that the myelin sheath of the affected nerves shows irregular swelling and fragmentation, but that the axis cylinder is not affected until polyneuritic symptoms appear: the changes resemble those occurring after section of a nerve, except that the alteration in the axis cylinder is considerably delayed. In the rat, H. H. Woollard (*Jour. Anat.*, vol. 61, p. 283; 1927) has only found lesions in the nerve-endings, both sensory and motor, of the voluntary muscles and in the intramuscular nerve fibres: they take the form of swelling of the nerve endings and fragmentation of the myelin in the sheaths of the nerve fibres, but the nerve axons remain intact. Similar, but less generalised, lesions occur in animals starved for a few days, and they can still be found even if vitamin B has been administered during the period of starvation. No lesions were observed in any other parts of the nervous system, central or peripheral.

It appears, then, that the lesions observed cannot be directly the cause of the symptoms, since they are too extensive to be abolished in a few hours, but they probably lead to a change in the axis cylinders or nerve-endings not demonstrable by present histological technique, which can be prevented or cured by administering the vitamin: the position is, however, rendered obscure by the fact that similar gross lesions are observed in starvation, and it is possible that starvation rather than vitamin B deficiency *per se* is the primary cause of both lesions and symptoms, the appearance of the latter, however, depending on the length of time the animal can live on the deficient diet, as compared with the short duration of a complete starvation experiment.

The actual amount of the vitamin required in the diet depends both on the diet and on the physiological condition of the animal. Thus Gladys A. Hartwell has shown that the rat requires about four times more vitamin B in the diet when rearing young than for growth or reproduction (*Biochem. Jour.*, vol. 19, p. 1074; 1925), whilst Drummond and Plimmer and their co-workers have demonstrated a relationship between the amounts of some of the other constituents of the diet and the vitamin B, which must be adhered to if optimal conditions for growth and maintenance are to be obtained (J. C. Drummond with Vera Reader, *ibid.*, vol. 20, p. 1256; 1926: and with A. Hassan, vol. 21, p. 653; 1927: R. H. A. Plimmer, J. L. Rosedale, and W. H. Raymond, *ibid.*, vol. 21, p. 913).

It is well known that the onset of symptoms of polyneuritis in birds is earlier in those which consume more of the deficient diet, and in those kept on a diet consisting chiefly of corbohydrates, whilst the onset is latest when the chief constituent of the diet is fat. Using young chicks or ducks, and, in the case of the carbohydrate diets, pigeons and rats also, Plimmer and his associates have succeeded in demonstrating a definite dependence of the vitamin B content of the diet upon the amounts of the other three chief constituents present if maximal growth and health are to be obtained.

The plan of the experiments was to feed diets consisting chiefly of protein, fat, or carbohydrate, together with salts and vitamins, and vary the relationship of the vitamin B to the main dietary constituent until satisfactory growth was obtained. The authors found that the ratio of dried yeast (as source of vitamin B) to the total calories in the diet was a constant and varied from 1 : 40 in the case of the chick, for a predominantly fat or carbohydrate diet, to 1 : 80 in the pigeon and 1 : 160 in the rat. Young animals require more than adults. The authors advance the suggestion that vitamin B is a constituent of the nucleus of every cell (perhaps a pyrimidine or purine compound), and thus the amount required depends on the total metabolism of the animal.

Drummond, working with rats, has observed that the ratio between the protein content and the yeast extract of the diet must be in the neighbourhood of 5, if growth is to be normal: with ratios of 18, growth was poor. He was unable to trace any relationship between the calories or the carbohydrate of the diet and the vitamin B content, thus failing to confirm the conclusions to which Plimmer was led by his experiments. The diet used by the latter for his rats contained no less than 94 per cent. of white flour, 5 per cent. fishmeal and 1 per cent. cod-liver oil completing it together with varying amounts of yeast extract: in none of Drummond's diets was the starch, as source of carbohydrate, higher than 70 per cent., nor did the protein content, in these experiments, caseinogen, fall below 20 per cent.: cod-liver oil, salts, lemon juice were also added, together with varying amounts of yeast extract, whilst fat sometimes replaced starch to increase the caloric value of the ration: in these differences between the diets used may lie the explanation of the somewhat different conclusions of the two observers.

That vitamin B really consists of two separate substances has been suggested by a number of different observations, but only recently has this differentiation become an established fact. Harriette Chick and Margaret H. Roscoe (*Biochem. Jour.*, vol. 21, p. 698; 1927) describe the two factors as the 'antineuritic,' which prevents polyneuritis in birds and paralysis in rats, and the 'pellagra-preventive' (of Goldberger), which prevents pellagra in rats, both together forming the water soluble vitamin B and both being necessary for normal growth. In a review of the literature, the authors point out that the two factors show certain differences in distribution; *e.g.* the antineuritic predominates in wheat embryo, but the antipellagrous in milk, meat, green leaves, etc., and that the antineuritic is more easily destroyed by a temperature of 120° C. and is more soluble in certain organic solvents such as alcohol, acetone, or benzene. In their experiments, the authors have confirmed the work of Goldberger and his associates on this subject.

The experiments were carried out on rats maintained on a synthetic diet: various preparations of yeast and wheat embryo were used as sources of the vitamins. On the basal diet alone, the animals died in a few weeks, usually without developing any symptoms: administration of dried yeast then permitted of growth and prevented death: if the yeast were first autoclaved, the animals died just as on the basal diet alone: if an alcoholic extract of yeast were given, the animals lived for some time, but without growth, and ultimately developed the symptoms of pellagra, characterised by ophthalmia, loss of fur, and dermatitis of the ears, paws, and neck. This condition could be cured, and at the same time growth restored, by the administration of autoclaved yeast. It is thus clear that the alcoholic extract of yeast contains the antineuritic factor, and the autoclaved yeast only the antipellagrous: growth occurs only when both are present together. The authors also confirmed the observation that wheat embryo contains mainly the antineuritic factor.

Hassan and Drummond have obtained similar results in their experiments (*loc. cit.*); thus rats kept on a high protein, low yeast, diet failed to grow satisfactorily until additional autoclaved yeast was added to the diet: but if only autoclaved yeast was used,

growth again failed to occur, but could be produced by the administration of small amounts of an alcoholic extract of yeast. The balancing of the protein in the diet appeared to be the function of the thermostable factor.

With this differentiation of vitamin B into two parts, greater knowledge of the chemistry of this vitamin should be soon obtained. Some light is thrown on the chemical nature and properties of the antineuritic factor by recent work by H. W. Kinnersley and R. A. Peters (*Biochem. Jour.*, vol. 21, p. 777; 1927, and by U. Suzuki and Y. Sahashi in Japan (*Scientific Papers, Inst. Physic. and Chem. Res.*, vol. 4, p. 295; 1926, and vol. 5, p. 191; 1927). Kinnersley and Peters have purified their yeast extract ('torulin') until only 0·15-0·3 mgm. per day is necessary to cure polyneuritis in pigeons.

The methods of extraction and purification used are briefly as follows: Yeast autolysed for three days at room temperature is extracted twice with boiling water: the combined filtrates are treated with neutral lead acetate, the precipitate removed and the filtrate treated with baryta. On filtration, a crystal clear yellow fluid is obtained containing 6000 doses of torulin from 14 lb. yeast. The barium is removed as sulphate and the filtrate treated with acid mercuric sulphate: after removal of the precipitate the reaction is adjusted to *p*H 7·0 and purified 'Norite' charcoal added. The charcoal adsorbs the torulin, which can be removed from it by extraction with hot 0·1 *N* hydrochloric acid, or with acid alcohol. About 60 per cent. of the torulin contained in the baryta filtrate is recovered. For prolonged feeding experiments, it is advisable to omit the mercuric sulphate stage: the solution can be cleared by 'Norite' charcoal, provided the *p*H is at 2·5, since at this reaction the torulin is not adsorbed, but remains in solution. Further purification may be affected by a prolonged alcohol fractionation, following removal of any traces of metals with hydrogen sulphide.

The purified material contains 15-25 per cent. nitrogen: it is soluble in absolute ethyl alcohol, but is not adsorbed on 'Norite' charcoal, like the impurer preparations, so that adsorption must be a property conferred on the torulin by some accompanying impurity. The Pauly reaction becomes less intense as the material is purified. The authors identify torulin with the thermolabile growth factor and consider that it is probably the same as the anti-beriberi vitamin. In view of the work previously discussed, the opinion that the antineuritic, antiberiberi, and thermolabile growth factors are the same substance appears to be justified at present, although future work may show the necessity of differentiating between them.

The Japanese workers have obtained a substance, which they call 'Oryzanin,' from rice-bran or yeast, which cures polyneuritis in pigeons in doses of about 5 mgm. The material was obtained from an alcoholic solution of rice-bran, by precipitating impurities with lead acetate and the active material with phosphotungstic acid, followed by silver nitrate. When boiled with dilute acids, the substance was split into glucose, choline, nicotinic and 2·6-dioxychinolin carboxylic acids. The latter has been found to have a stimulating effect upon the growth of yeast, whilst a closely related compound, 2·6-dioxychinolin hydrochloride, in doses of about 7·5 mgm. daily, injected intramuscularly, cures the polyneuritis of pigeons, but has no influence on their weight. The authors consider that these compounds are closely related to the active principles contained in rice-bran: caution must be exercised, however, in accepting this conclusion, since the substances isolated might be contaminated with minute traces of a very highly active compound, in the same way as the activity of irradiated cholesterol has been shown to be due to contamination with small amounts of ergosterol, the latter only being converted into vitamin D on irradiation.

The work reviewed above offers hope that the designation 'vitamin B' will soon have to be discarded in favour of the proper names of well-defined chemical compounds, and that their isolation will be followed by their synthesis in the laboratory.

Biology of the Gulf of Mannar.

THE recent *Bulletin* of the Madras Government Museum, edited by the Superintendent (The Littoral Fauna of Krusadai Island in the Gulf of Mannar. New Series. Natural History Section, vol. 1, No. 1. 1927. P. 196. Madras: Government Press. 8 rupees), is the first issue of a new series of Madras Government publications on the natural history of animals and plants, as distinct from a general section dealing with archæology, anthropology, and allied subjects. The treatment of the subject in this number is one intermediate between a text-book and a specialist's monograph. Descriptions of many common species of invertebrate animals found on Krusadai Island in the Gulf of Mannar and in neighbouring localities are given. The classification followed is either that of the "Oxford Zoology" or of the "Cambridge Natural History." A new genus is described, Pseudocaprellina, in the suborder Caprellidea. There are two appendices, the first on the vertebrate fauna and the second on the flora of the island.

The various authors, among whom Dr. F. H. Gravely and Dr. B. Sundara Raj are prominent, have adopted a useful system of describing the common species, of which no up-to-date account exists within the reach of the Indian student. They have illustrated their papers well and given good lists of literature under each group. This publication will be valuable to students not only as a preliminary guide to the fauna and flora of the Island, but also as a stimulus to collecting and to subsequent research in the biological world of India.

The southern side and the eastern part of the northern side of the Krusadai Island are entirely sandy; the western part of the northern shore is very muddy and fringed with mangroves. A salt marsh, bounded on its southern and eastern sides by high sand dunes, extends from the northern shore across the whole width of the island, a little to the east of the middle. Tolerably fresh water can be obtained from shallow pits dug in the sand. Swarms of anopheline larvæ have been found, but no mosquitoes have been seen in September or in April-May.

From this island there is an easy approach to collecting grounds in Shingle Island, Kutikāl Point on Rāmēswaram Island, Pāmban Channel, and Rāmēswaram. Shingle Island gives ample opportunities for observing corals in their natural surroundings; Kutikāl Point is a good place for collecting medusæ and other pelagic forms; the Pāmban Channel is extraordinarily rich in hydroids, polyzoa, ascidians; and Rāmēswaram is interesting, as its fauna is very different from anything yet found in the immediate neighbourhood. With such surroundings and the prospects of the establishment of a Marine Biological Station in Krusadai Island, there is every hope that much information of interest on tropical marine life will be forthcoming in the near future.