Mineral Metabolism: Iodine and Sulphur.

THE importance of the mineral salts in nutrition is sometimes obscured by the greater attention devoted to other dietary constituents, more especially within recent years to the vitamins; but, of course, they are just as important for life as any of the other irreplaceable elements of the diet. The functions of the chlorides of sodium, potassium, and calcium in maintaining the osmotic equilibria between the fluids and cells of the body, without which the latter cannot function normally, the necessity of a supply of calcium and phosphorus for the formation of bone, and of iodine for the production of thyroxine, and the part played by the sulphur-containing amino-acid, cystine, in growth, all indicate the importance of studies of the mineral metabolism.

The functions of iodine and sulphur in the animal economy offer some interesting analogies as well as points of contrast, and have recently been reviewed by Orr and Leitch,[1] and Marston and Brailsford Robertson,[2] respectively. Our knowledge of the metabolism of these two elements, although extensive, is still incomplete at many points, but critical reviews are of value by indicating both the present position of our knowledge and also the points at which further work may be at the moment most profitably carried on. The present article will summarise briefly some of the more salient features of the positions of iodine and sulphur in nutrition and metabolism.

IODINE.

It is of interest to note that the work of the first quarter of the twentieth century has been largely a repetition of that carried out during the first half of the nineteenth, with the result that the earlier conclusions have been essentially confirmed, although the investigations were performed with few of the modern facilities for accurate research work which we now possess.

Iodine is very widely distributed in Nature, but it is usually present in only minute amounts. Even in its richest sources, such as saltpetre deposits and sponges, its maximum concentration does not exceed 0·1-0·2 per cent. In other minerals or animal tissues the amount present may be reckoned in thousandths of a milligram. It has been found in all geological deposits examined in concentrations varying from 170 γ to 9200 γ per kgm. ($\gamma = 10^{-6}$ gm.); in soils it occurs in quantities varying from 600 γ to 6000 γ per kgm.; it tends to be absorbed by acid soils and is concentrated by the plants growing on the soil, both processes leading to enrichment of the soil. On the other hand, depletion occurs in basic soils or those rich in calcium and also in heavily cropped soils. Water contains much less iodine than soil; fresh, 1 γ per litre, and salt, 17-18 γ per litre; marine plants and seaweed concentrate the iodine from sea water and form rich sources of the element. Only traces are found in salt deposits or in air.

It thus appears that plants must provide the main source of iodine for animals, and it is of importance to know something of the factors affecting the iodine content of the former. The available iodine in the soil, which may not be the same as the total iodine, is of much greater importance in determining the iodine content than the species to which the plant belongs; but the actual part of the plant in which the concentration is highest varies with the species. Sea plants contain the most iodine and land plants the least, with freshwater intermediate. The effects of supplying additional iodine for plant assimilation depend on the dosage used, the species of plant, and the medium to which it is added. In general, it may be stated that with the amount of iodine available at its optimum level, growth is at its maximum, and the iodine concentration and nitrogen assimilation are increased; the storage of useful products, for example, sugar in the sugar-beet, is improved, and there is also an increase in the aerobic respiration, and in the *p*H of the cell sap; the activity of nitrifying bacteria is stimulated. Doses greater than the optimum are toxic, but the amount required to produce such an effect varies with the species of plant studied.

In animals, the iodine content of the thyroid gland reaches 0·1-0·2 per cent or more of the dry weight, but the concentration in the other tissues of the body is much less. At present the only known function of iodine in the animal economy is in the formation of thyroxine, a stimulator of metabolism and essential for growth and health. In addition to thyroxine, the thyroid gland also contains di-iodo-tyrosine, and it appears probable that these two compounds account for the whole of the iodine content of the gland (C. R. Harington and S. S. Randall: *Chem. and Indust.*, vol. 48, p. 296; 1929). The latter is influenced by a number of factors, but depends chiefly on the intake of iodine and less upon the age, sex, or species. The differences between individuals of the same species are as wide as any differences between species, except that the glands of sea fish contain up to 1·6 per cent iodine (dry weight), whilst those of rats appear to have a low content, 0·06 per cent (dry weight) having been reported, but these differences are probably explicable by varying levels of intake. In normal glands the total iodine content increases with the age of the individual as the gland grows to its maximum in adult life, the iodine percentage remaining fairly constant; only in the very young fœtus is the percentage lower. In some animals the iodine content is greatest in autumn, coincident with an increase in the iodine content of the pasture; the percentage also varies inversely with the

[1] Medical Research Council. Special Report Series, No. 123: Iodine in Nutrition; a Review of Existing Information. By J. B. Orr and I. Leitch. Pp. 108. (London: H.M. Stationery Office, 1929.) 2*s*. 6*d*. net.

[2] Commonwealth of Australia: Council for Scientific and Industrial Research. Bulletin No. 39: The Utilization of Sulphur by Animals, with Especial Reference to Wool Production. By H. R. Marston and T. Brailsford Robertson. Pp. 51. (Melbourne: H. J. Green, 1928.)

weight of the gland, but this is only true of normal glands.

The concentration of iodine in other tissues is low, for example, 5-15 γ per 100 c.c. of blood, rising in women at the beginning of menstruation, doubtless due to change in the physiological activity of the thyroid gland with the sexual cycle. The body tissues generally account for 40-80 per cent of the total iodine, according to the species, the remainder being in the thyroid gland.

The effects of the administration of iodine to animals are in many ways similar to those produced by feeding thyroid gland itself, since the iodine may modify the gland's secretion. Both inorganic and organic iodine occur in the blood; the former is passing to the gland or results from the disintegration of thyroxine in the cells of the body. The latter is probably chiefly thyroxine itself, on passage to the tissues of the body, since its amount is decreased after thyroidectomy. It may be pointed out here that inorganic iodine can be readily assimilated by the body, whereas inorganic sulphur cannot be utilised, only organic compounds of the latter being taken up and metabolised.

Iodine may produce increased growth in animals and in children in goitrous areas, but such a result appears to be presumptive evidence that the intake was previously below the optimum. On the other hand, thyroid administered in excess can inhibit growth, acting as a toxic agent. Small doses of iodine or thyroid increase the retention of nitrogen, whilst the larger doses of the latter increase its output; coincident with these effects, thyroid administration stimulates the general metabolic processes of the whole body, whilst small doses of iodine may, under certain conditions, decrease these, at the same time decreasing the organic iodine in the blood.

Light has been thrown on the physiology of the thyroid gland by studies of its diseases. A healthy gland stores colloid in its acini, but this only occurs where the iodine content is about 0·1 per cent of the dry weight, or 0·03 per cent of the fresh weight or more; hence an adequate intake of iodine is essential for the proper functioning of the gland. In both simple and exophthalmic goitre the percentage of iodine is subnormal. The variations in the supply of thyroxine to the tissues are reflected in alterations in the concentration of iodine in the blood.

Data from metabolism experiments and the average iodine intake in non-goitre as compared with goitre areas indicate that the minimum daily amount required for equilibrium is, in the adult, 15 γ, and in the child, 50 γ, but to allow a liberal margin of safety, at least three times these quantities should be consumed. Endemic goitre can be prevented and usually cured by supplementing the iodine intake, and it has been found that about 100 γ daily are required; this amount can be conveniently obtained by using salt to which a small dose (1 : 200,000) of potassium iodide has been added.

To sum up, animals can obtain the iodine they require from such a source as an inorganic iodide, or iodine itself may be administered; usually the requisite quantity is consumed with the animal or vegetable food eaten, partly in inorganic and also probably partly in organic form, but little is known of the organic iodine compounds in plants. In animals the iodine is taken up by the thyroid gland, for the formation of thyroxine, di-iodo-tyrosine presumably being an intermediate stage. The thyroxine has a profound influence upon the metabolism of all the cells of the body; although after its administration in even a small dose, the stimulating effect is prolonged, yet the continual loss of iodine from the body indicates that the thyroxine is continually destroyed, with the result that a steady supply of iodine is necessary to maintain a constant formation of the hormone. In the absence of a sufficient absorption of this element, the thyroid gland enlarges, and finally may fail to supply sufficient thyroxine for the needs of the body, producing a slowing of metabolism in the adult, and also, in the young, failure of growth.

SULPHUR.

In contrast to iodine, animals can only utilise organic sulphur, inorganic forms being treated as waste products and excreted. Hence they are dependent upon plants or other animals for their supply of this essential element. Plants, however, can utilise sulphur and sulphates, and their addition to soils poor in sulphur increases not only the content of this element, but also the assimilation of nitrogen, indicating presumably the formation of sulphur-containing amino-acids.

Until recently, only one organic compound containing sulphur was known which is assimilable by animals, the amino-acid cystine. Natural cystine is lævo-rotatory and differs in its properties from its optical isomer and the racemic form. The cystine content of proteins, both vegetable and animal, is usually low, varying from 0·5 to 3·0 per cent, but it is still lower in gelatin and casein; wool keratin, however, contains about 13 per cent, and the protein from *Antiaris toxicaria* upwards of 25 per cent. Part of the sulphur in proteins can be split off as hydrogen sulphide by heating with alkali, suggesting that two forms of sulphur exist in the molecule, and therefore that cystine is not the only sulphur-containing amino-acid present. More recent work, however, has demonstrated that sulphur in diketo-piperazine linkage is very labile to alkali, and the two forms of sulphur disclosed by this reagent in the molecule may simply indicate cystine in diketo-piperazine and in catenary linkage respectively.

A second sulphur-containing amino-acid has been isolated from caseinogen and some other proteins in amounts of less than one-half per cent, and Barger and Coyne have recently succeeded in synthesising it and determining its constitution; it is γ-methyl thiol-α-amino-butyric acid, $CH_3 . S . CH_2 . CH_2 . CH(NH_2) . COOH$, and has been called 'methionine' (G. Barger and F. P. Coyne, *Biochem. Jour.*, vol. 22, p. 1417; 1928). The

starting-point in the synthesis was β-methylthiolpropaldehydeacetal formed from methylmercaptan and β-chloropropaldehydeacetal; from it the aldehyde was obtained and then γ-methylthiol-α-aminobutyronitrile, from which the amino-acid was easily formed.

The sulphur-containing compounds occurring in the body, for which the intake of sulphur is required, are glutathione, taurine (in taurocholic acid in the bile), chondroitin or mucoitin sulphuric acid (in mucoproteins), and sulphur-containing lipoids: whilst inorganic and ethereal sulphates occur in the urine as oxidation products of the above, with thiosulphates, thiocyanates, taurine, and cystine. In addition, oxidised sulphur is utilised for conjugation with various compounds foreign to the body tissues which might otherwise exert toxic effects.

Deficiency of cystine in the diet of a young animal is reflected in imperfect growth: thus 18 per cent of the protein phaseolin (from the kidney bean) in the diet of the young rat will not support normal growth unless 2 per cent of cystine is added. Again, adult mice will not live on diets deficient in cystine content: inorganic sulphates, sulphur, dithioglycollic and dithiopropionic acids, cysteic acid, and taurine cannot replace the cystine, but dipeptides of the amino-acid are utilised. The loss of sulphur in the urine indicates the necessity for a continuous intake of this element: mucus is also being continually lost from the body and a certain amount of taurine is excreted, although the greater part of the bile acids is reabsorbed from the gut and again excreted in the bile. Our present knowledge suggests that the necessity for a constant supply of glutathione in the cells of the body is the main factor controlling the requirement of cystine, both in maintenance and growth.

Attempts have been made to trace the paths of cystine metabolism by adding it or other sulphur compounds to the diet: by feeding compounds of cystine in which radicles have been added to the amino or sulphhydryl groups or both together, it has been shown that for oxidation to occur, it is necessary that these two groups should be free. Again, from the fact that ingested taurine is excreted unchanged, it appears that deaminisation must precede oxidation of the sulphhydryl group in normal metabolism. These processes probably occur chiefly in the liver, and the three carbon chains left after removal of the sulphur and nitrogen are either converted into protein or glucose (as in the phloridzinised dog) or oxidised completely.

The proportion of ingested cystine retained as such to that oxidised and excreted will depend on the needs of the tissues and on the form in which it is supplied. Greater retention occurs when cystine is given in a combined form, even as a dipeptide, than when administered in the free state, and it is probable that lævo-cystine is better utilised than the racemic form. In fact, the toxic influence on the kidneys of small quantities of cystine is probably due to the fact that the dextro or racemic form has been given, since larger amounts in natural combination in protein are without this effect.

The blood contains sulphur compounds on their way to and from the tissues: in man, about half the sulphur is present as sulphate, inorganic or ethereal, the other half being 'neutral' sulphur and consisting of glutathione (0·1 per cent) and ergothionine, the betaine of thiolhistidine (0·01-0·025 per cent). The physiological function of the latter compound is not known.

Taurocholic acid occurs in much higher concentration in the bile of carnivora than in that of herbivora, but during protein starvation the amount is reduced and glycocholic acid takes its place. In the dog, cystine will not increase the amount of taurocholic acid without the simultaneous administration of cholalic acid, but in the rabbit cystine alone is the limiting factor, its administration resulting in abnormally high concentrations of taurocholic acid in the bile. Free taurine is not further metabolised by the tissues, but in herbivores its administration by mouth results in an increase in the sulphate excretion, since it is decomposed by the bacteria in the large intestine.

Much work has been carried out on the detoxicating effect of oxidised sulphur compounds. The ethereal sulphate of the urine is chiefly potassium indoxyl sulphate and represents the end point of bacterial decomposition of tryptophane in the intestine: the tissues destroy the amino-acid by a different path, since it is completely burnt to its fully oxidised end products. Similarly, administered phenol is excreted in combination with sulphuric acid, but aromatic amino-acids are completely oxidised, so that phenol cannot be in their path of degradation. Certain benzene derivatives are excreted as mercapturic acids in the dog, for example, bromo- or chloro-benzene, but such synthesis probably does not occur in the pig or in man. Phenol and ortho-, meta- and parachlorphenol do not give rise to mercapturic acid formation (see T. S. Hele and co-workers: *Biochem. Jour.*, vol. 20, pp. 598 and 606; 1926: vol. 21, pp. 606, 611, and 628; 1927). When mercapturic acid formation does occur, an abundant supply of cystine is required: this is obtained by feeding the dog on a high protein diet or injecting cystine, but on a low protein diet its formation is much decreased.

Apart from the necessity for a supply of cystine for growth and maintenance, this amino-acid may have an economic importance and become the limiting factor in the suitability of a pasture for the raising of sheep for wool production, since the protein of wool contains upwards of 13 per cent cystine, whilst the food proteins probably contain only 1-2 per cent. Finally, iodine may also become the limiting factor under certain conditions, since there is evidence that thyroid feeding will improve hair growth even in under-nourished animals and at the expense of a further loss of body weight, indicating that a plentiful supply of thyroxine is necessary for hair growth as well as body growth, and that a deficient intake of iodine may decrease the production of wool even if the cystine intake is adequate.