

MINERAL AND VITAMIN REQUIREMENTS AND THE WAR-TIME DIETARY

AT a discussion in the Section of Medicine of the Royal Society of Medicine on May 26 on mineral and vitamin requirements and the war-time dietary, some interesting and important points were brought out, though the subject is very wide and could therefore be covered only in part because in the last limit it embraces both the qualitative as well as the quantitative requirements.

Mineral Requirements

Dr. R. A. McCance confined his attention mainly to calcium and iron, saying that sodium, potassium, magnesium, zinc and phosphorus and the 'trace elements' such as copper and manganese can be left to themselves. He opened by stating that to maintain ourselves in perfect health we have to *absorb* just as much calcium, phosphorus and other mineral elements as before the War; even then there was a belief that calcium and iron were deficient. In the modified and planned war diets, there is now plenty of milk for children but little for adults. The calcium and iron intake is maintained by the 85 per cent extraction loaf which has 2-3 times as much iron as in white bread. The problem is whether these two minerals are as well absorbed as in peace-time. In an investigation by Drs. McCance and Widdowson upon human subjects (taking more than fourteen months) the calcium proved to be less well absorbed when brown 92 per cent extraction flour was substituted for white flour in the diet. The same has been found more recently with iron, though the results need some repetition. The nature of the factor responsible for depression of absorption has emerged as the result of much research. E. Mellanby earlier stressed the presence of a rachitogenic factor in some cereals, especially oatmeal. Much later (1934) Bruce and Callow suggested that this might be inositol hexaphosphoric acid, a compound present in the outer parts of the cereal grains. In 1939 Harrison and Mellanby showed that addition of sodium phytate to puppies' diet led to rickets. It was left to McCance and colleagues to prove that the addition of sodium phytate to white bread depressed absorption of calcium and magnesium more than the brown bread diets, some subjects even excreting more calcium in the faeces than they took in the food (due to fixation of calcium in some of the intestinal secretion). Removal of phytin in bread is possible on the experimental scale though impracticable commercially, and does not completely reduce the error in absorption. Nor does vitamin D in physiological doses improve the absorption of calcium in adults. The cheapest and simplest way to counteract the action of the phytic acid is to add calcium as calcium carbonate to precipitate the phytic acid and so leave the remaining 'calcium' available for absorption. In the 85 per cent loaf this is about half the amount of phytic acid present in the '92 per cent'. Dr. McCance considers that the physiological arguments for addition of calcium (as calcium carbonate) to the 85 per cent loaf are very strong and that we should have the courage of our convictions and support the addition to maintain adequate calcium absorption. A similar argument holds for iron.

Prof. R. A. Peters in a short communication pro-

duced X-ray pictures by Dr. Kemp, of Oxford, to demonstrate that in a man of 52-53 years the addition of calcium carbonate to the maximum amount recommended by Dr. McCance and adopted by the Medical Research Council (namely, 14 oz. per 280 lb. flour) to 85 per cent extraction bread has produced no signs either of calculus or calcification of the femoral arteries. His family has lived on this bread (+ chalk) for thirteen months with no noticeable ill-effects, as indeed would be expected, when it is realized that the addition is only 1/20 oz. calcium carbonate in 1.3 lb. bread.

Dr. Murray Scott discussed minerals from a practitioner's point of view; as chairman of a local borough committee on bread, he has been trying to get evidence of deficiencies attributable to faulty diet, etc. In his part of the country medical and obstetrical opinion asserted there is increasing anaemia; examination of miners' wives showed reduction in haemoglobin, and indicated that the miner frequently eats all the meat and cheese in the family ration. For several years second-year medical students at Leeds have estimated their own haemoglobin; for the six years before the War the numerically largest group has always been in the 100-105 per cent haemoglobin division. Last year, it was the 90-95 per cent division. A careful survey of prices in August 1941 showed that for an active man a complete optimum diet could not be purchased for less than 14s. a week, and for sedentary workers 11s. per week. Since the rationing of meat, bacon, eggs and cheese reduces the intake of iron to 1-2 oz. a day from this source, there is a theoretical expectation of increasing anaemia among the poorer half of the nation. The change to 85 per cent extraction meal will increase the iron of the diets for most people by about 2.5 mgm. a day, without disturbing the supply of calcium and phosphorus which comes from the milk, though the use of some of the wheatings in the new flour will deprive pigs and poultry of part of their present supply. In considering possible disadvantages of 85 per cent extraction flour, Dr. Scott was struck with the apparent discrepancy between the conclusions of Widdowson and McCance's experiments on iron and his general ideas, and wished to have evidence of lack of energy or fall in haemoglobin. He instanced the case of two adults in his own household, who have eaten entirely wholemeal bread, and yet have haemoglobins of more than 100 per cent. How does their iron escape the clutches of the phytic acid? In a speculative vein, he asked whether it is possible that prolonged eating of white bread led us to lose a capacity to deal with phytic acid (by phytases or otherwise) which was possessed by our ancestors. In conclusion, he expressed the view that diets contain less total iron than pre-war and that it is important to solve the question of how to increase the available iron in the nation's diet, preferably through bread.

In the parts of the subsequent discussion which bore upon minerals, Dr. H. M. Sinclair pressed for supplements of iron in the national dietary. Dr. A. P. Meiklejohn, in dealing with the question whether the addition of iron to bread was justified upon the evidence so far produced, stated that in a recent survey in Lancashire a random sample of young married women had (low) haemoglobin limits of about 75 per cent (Haldane scale). Dr. Geoffrey Evans was impressed by the number of persons who had haemoglobin values below 85 per cent and the obvious improvement by giving iron.

Prof. J. R. Marrack was a sceptic upon the possible 'de-mineralizing' effect of brown bread, as he has always eaten it and been able to play rugby football until he was forty-four. Dr. R. J. Atkins referred to the importance of trace elements and to the presence in soil of elements like manganese which interferes seriously with the absorption of iron. Mr. A. L. Bacharach said that in order to increase the general intake of iron by 5 mgm. per person a day, only eighty tons of iron salts would be needed, and that it was quite easy to carry this out with bread.

Dr. McCance, replying to several points, pointed out that the calcium requirement of individuals shows large variation; some would store calcium on a diet which would lead to loss of calcium in others. This probably explained Prof. Marrack's point. It makes the postulate of an absolute average requirement difficult, and indicates the need for playing for safety.

Summarizing this side of the discussion, there seems to be a general belief in the need for calcium and iron supplements in the diet, in some form, of which bread appears a reasonable medium.

Vitamin Requirements

The requirements of vitamins in the dietary were less thoroughly discussed, the main attention being given to vitamin C. Dr. L. J. Harris urged the existence of subclinical deficiencies. He directed attention to animal experiments in which it could be demonstrated that there is a grading of response to vitamin B₁ between the optimal dosage-level and one giving definite signs of deficiency. It is unlikely that this does not apply to man. He considers that his investigations have shown that levels of nutrition of some vitamins could be judged quantitatively by statistically controlled 'saturation tests' upon groups of people; this must not be taken to mean that he thinks that perfect nutrition is only co-existent with saturation. In the urinary saturation tests for vitamin C, the level of nutrition is judged by the speed with which the urinary excretion with test doses reaches on average a plateau; this takes only one or two days in subjects receiving proper amounts of the vitamin in their diet. Absence of scurvy does not mean necessarily having enough vitamin C. There occurs in man those symptoms of deficiency seen in guinea pigs, namely, diminished growth-rate, failure in the production of new tissues and increased liability to infection. He cited work from German official records in support of the view that extra vitamin C improves the annual gains in weights and height of children and diminishes the incidence of infections (1,600,000 children were given 50 mgm. vitamin C daily in addition to the ordinary war-time diet). In Britain, Glazebrook and Thomson have found less duration of tonsillitis and less disease in naval trainees dosed with vitamin C. Further, Crandon and associates in America and Hunt in Great Britain believe in the value of vitamin C in the healing of wounds; but it is true that the effect upon Crandon himself did not appear until a late stage in his vitamin C deficient diet. On the other side he quoted the view of Zilva (an authority on vitamin C) that even allowing for destruction in cooking, the war-time diet contains enough vitamin C, and also the observations of Fox and colleagues; the latter found that out of 950 miners getting 12-25 mgm. of ascorbic acid twelve developed scurvy, whereas only one out of a similar number getting an extra 40 mgm. developed scurvy.

This point was taken up by Dr. Sinclair, who thought that it created much difficulty in regard to subclinical deficiency, because there was no difference in incidence of infection in Fox's experiments. On the other hand, Dr. Meiklejohn said that long before Dr. Crandon showed signs of vitamin C deficiency he looked a sick man and suffered from vague symptoms of lassitude.

Dr. Harris also referred to the results of some of his saturation tests. Children at a good residential institution showed consistently higher levels of excretion of vitamin C than those from poorer homes; but during the War the level of both groups has fallen; this was more marked in the winter months and justified the decision to set aside supplies of vitamin C for young children.

In relation to this, Prof. Marrack said that in March and April he estimated ascorbic acid in a number of meals in school canteens and British Restaurants, where he found no evidence of excessive destruction in the preparation and cooking. The total ascorbic acid calculated as supplied for one third meals was less than 10 mgm. and for one third was more than 20 mgm. There was evidence that in saturation tests the number of boys saturating on the first day of giving the test dose increased with the average ascorbic acid content of the diet at the school. The highest saturation was in the school which grew abundant early green vegetables, and the choice of vegetables grown is important. It is unwise to trust to raw vegetables.

Dr. Atkins raised some critical points over the saturation tests. He found that in fifty men from the same kitchen differences of saturation occurred according to whether they had their vitamin C tablets before or after breakfast. Further, 40-100 left saturated ten months ago, showed no quicker saturation in a subsequent period of dosing than those previously unsaturated.

In conclusion of his remarks, Dr. Harris said 9 per cent of children in an institution, 15 per cent among urban children, and 18 per cent among village children could be improved by a vitamin A dosing, using a dark adaptation test (Yudkin). Six per cent of school children examined at Cambridge showed riboflavin deficiency (vascularization of the cornea by slit lamp examination). Dr. Geoffrey Evans from his own experience believes in the existence of deficiencies to-day, and stated that cases of acne rosacea with conjunctivitis and mild degrees of keratitis could be improved by giving riboflavin, and cases of fatigue and depression with vitamin B₁. Dr. Magee, striking a critical note, considered that not enough attention had been paid in the discussion to the quantitative requirements and pointed out the great differences between the standard for proteins and minerals which had been arrived at as the result of clear-cut experiments, and the vitamins. For these he regarded the evidence as still more approaching guess-work or speculation.

It is the opinion of the reviewer that the problem of actual quantities of vitamins required could well be discussed again, when more information is available. In regard to this vexed question of 'sub-clinical deficiency', it must be conceded that evidence is accumulating that levels of saturation of some vitamins may fall to a point which is dangerous in the sense that individuals may be too close to the point of actual deficiency. Such depletion periods will not matter so long as they are only biochemical, provided that they are reversed in time, because

there may be still enough vitamin about to run essential biochemical processes. If, however, there is also the ill-health suggested, it is important; but its very vagueness must increase the need for caution and for prolonged experiments (taking into account all psychological factors) before coming to a final judgment. It is difficult to see how the state of the individual (as distinct from statistical averages over a group) can be assessed without combining blood estimations with clinical examinations, as Dr. Sinclair and his colleagues are trying to do. So much for the purely scientific attitude. Meanwhile we are faced with practice in the War. We know from McCance and Widdowson that there is a wide difference in calcium requirements of individuals; we have the League of Nations standards for the earlier vitamin factors, but none for those more recently discovered; we know that even animals are individual in their needs of a vitamin like aneurin. Let us hope that ultimately we may be able to recognize on a scientific basis cases where intake of a special factor must be increased for an individual. For the moment we are left with the dilemma that diets must provide sufficient margin of accessory factors to ensure no physical inefficiency, and we must see that time and valuable foodstuffs (synthetic and natural) are not wasted; fortunately, the latter does not apply to chalk and iron.

R. A. PETERS.

AMINO ACID ANALYSIS AND THE STRUCTURE OF PROTEINS*

By PROF. A. C. CHIBNALL, F.R.S.

IN recent years X-ray crystallographers have made remarkable advances in the interpretation of protein structure, and it is becoming more and more evident that a stage has been reached when their views need to be reconciled with data obtained from accurate amino-acid analysis of the proteins concerned. In 1934 Astbury suggested that in gelatin the residues of glycine and hydroxyproline must repeat at regular intervals along the peptide chain, an idea developed in the following year by Bergmann and Niemann into a general hypothesis that (briefly) the 'frequency' of recurrence of any particular residue must be factorizable entirely by 2 and/or 3, as must also the total number of residues in the molecule. Evidence in its favour was adduced from analytical data for several proteins, including egg-albumin. The hypothesis has been criticized on various grounds, chief among them being that existing methods of analysis are in most cases not sufficiently quantitative to warrant the results being interpreted with the precision needed. What are believed to be quantitative estimations for the bases and dicarboxylic acids in edestin, egg-albumin and β -lactoglobulin have now been obtained.

Edestin.—The analytical data and calculated mean residue weight (115.7) show that the smallest possible unit contains 432 ($2^4 \times 3^3$) residues, and that the number of residues of arginine, histidine, lysine, tyrosine, tryptophan, methionine and cystine are 48, 8, 8, 12, 4, 8 and 3 respectively. On the assumption that the amide-nitrogen can be distributed in such a way that the number of residues of asparagine, aspartic acid, glutamine and glutamic acid are also factors of 432, it can be shown that these must be 27, 18, 36

and 36 respectively. The data lend support to the Bergmann and Niemann hypothesis, but at present there is no evidence that edestin is homogeneous.

β -Lactoglobulin is homogeneous. The analytical data suggest a molecular weight of 42,000, in agreement with physical measurement, but the total number of residues is not factorizable by 2 and/or 3. The Bergmann-Niemann hypothesis is thus not valid. On the assumption that the molecule is one peptide chain, the number of carboxyl groups computed from titration curves is in agreement with the chemical data, but the number of free amino groups is eight more than can be attributed to lysine. The molecule is a system of nine peptide chains, not all of like composition, coupled by linkages involving carboxyl and not amino groups. Imide and thio-ester linkages are suggested; the presence of the latter would explain many puzzling features of denaturation.

Egg-albumin is not homogeneous and tells a somewhat similar story. Chemical data suggest a molecular weight of 43,000, in conformity with physical measurements; the Bergmann-Niemann hypothesis does not hold. From titration and other data it is suggested that the molecule is a system of at least four peptide chains having free terminal amino groups, recalling Astbury's deduction that the denatured protein is a pile of four laminae.

Insulin.—If the excess of free amino groups is a measure of the number of peptide chains, as in lactoglobulin, the molecule of insulin must be a system of eighteen such chains, a striking confirmation of Bernal's prediction from the peaks in the Patterson projection.

Reverting to *edestin*, it has been found that the determination of free amino groups is complicated by edestan formation. It is probable that the sub-molecule of 50,000 is one peptide chain, though the possibility that it is a system of three peptide chains of unlike composition cannot be dismissed, in which case the allocation of the amide-nitrogen given above would not be justified. Nevertheless, the stoichiometric relationships exhibited by the other residues are sufficiently impressive to warrant the assumption that the constituent peptide chains would be built to essentially the same pattern based on $2^n \times 3^m$, though they might not contain the same number of residues. All other proteins may be similarly constituted. Such views emphasize the need for more work on the isolation and characterization of the partial decomposition products of protein, for much of the evidence hitherto brought forward to support the Bergmann-Niemann hypothesis has been based on inadequate experimental data and has demonstrated nothing more than the hypnotic power of numerology.

TIMBER-SEASONING KILNS

TWO leaflets have recently been issued by the Forest Research Institute, Dehra Dun, India, on the subject of timber seasoning kilns by M. A. Rehman, Utilization Branch (For. Res. Inst. Public. Vasant Press, Dehra Dun, 1941-42). Leaflet No. 11, "Types of Timber Seasoning Kilns suitable for drying Indian Woods" (1942), describes briefly the various types of seasoning kilns which have been designed or tested by the Forest Institute. It was due to the position in which we found ourselves during the last War in the matter of timber supplies, especially

* Substance of the Bakerian Lecture delivered before the Royal Society on July 16.