CXIX. HYPERVITAMINOSIS AND VITAMIN BALANCE.

IV. AN INSTANCE OF VITAMIN BALANCE.

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THE object of this paper is to record a continuation of experiments in which the existence is demonstrated of a "balance" between the vitamin B complex on the one hand and vitamins A and D of cod-liver oil on the other. Only two previous communications bearing directly on this subject have appeared in the literature, so far as we are aware. In the first place, Hopkins [1923] mentioned that he had found that a moderate excess of cod-liver oil was often injurious to the rat unless "balanced" by an increased marmite (vitamin B) allowance¹. Secondly, Harris and Moore [1928] have published details of investigations in which it was shown that the substitution of cod-liver oil for arachis oil in rations containing various restricted allowances of vitamin B resulted in a further lowering of growth rates².

In these experiments comparatively large quantities of cod-liver oil (15 % of the diet) were administered, and, since there are many substances in cod-liver oil of whose chemical nature and properties little is known, there was no necessity to assume that the ill-effects observed were necessarily due to vitamins. (Our conclusions here were contrasted with those concerning the specific hypervitaminosis D resulting from excessive irradiated ergosterol.) It was therefore of interest to repeat our experiments using vitamin concentrates instead of cod-liver oil so as to eliminate at least the materials present in the saponifiable fraction of the oil.

EXPERIMENTAL.

Carefully matched sets of young rats from split litters have been used for this work which has been repeated in duplicate using both albinos and piebalds. Similar synthetic basal diets were fed to all sets, viz. purified "casein" (J. Nathan

¹ We are permitted by Sir F. G. Hopkins to make it clear that his work was in fact concerned with high cod-liver oil diets as here indicated, and not with low cod-liver oil diets as the printed report of the Cameron lecture would make it appear.

² Apart from the question of vitamin balance, injurious effects produced by cod-liver oil have also been described by a considerable number of workers: a bibliography is given in our earlier paper.

and Co.) 20 %, arachis oil 15 %, rice starch (British Drug Houses) 60 %, salt mixture 5 %, but the sets differed in receiving various graded allowances of vitamin B (e.g. in Fig. 1, 1 %, 2 %, 4 %, 8 %, 16 %, 32 % of our standard marmite extract [Harris and Moore, 1928]). Each of these sets was further divided into several sub-groups, and to each of these a different level of concentrate¹ was administered (viz. in Figs. 1 and 2, 2 mg., 10 mg., 50 mg. and 250 mg. of concentrate per 10 g. of basal diet, the concentrate being dissolved in the arachis oil as a constituent of the diet). It was thus possible to compare the effects upon the animals of variations either (1) in the marmite allowance at each level of concentrate, or (2) in the concentrate allowance at each level of marmite.

Results. The results obtained have been distinguished by a remarkable regularity in the retarding action of the vitamins A and D concentrate upon growth rates. This action has been found, in fact, to be no less regular than the inverse accelerating action of marmite, and in no case (except at totally inadequate levels of marmite) has any growth curve fallen out of its predicted position.

1-2 % *Marmite*. At these low levels of vitamin B, known to be insufficient for maintenance even in diets containing normal amounts of vitamins A and D, moderate excess of concentrate (0.1 %) did not appreciably alter the rate of decline (Fig. 1)².

3.2-4 % *Marmite* (Figs. 1 and 3). At levels of vitamin B representing border-line concentrations in normal diets even moderate excess of concentrate could become the deciding factor in the fate of the animals. Thus in Exp. 3, Fig. 3 (3.2 % marmite) the addition of 0.1 % of concentrate to the diet induced early failure in growth, decline in weight, and eventually a dying condition. While the rats receiving concentrate became hunch-backed, rough coated and partially paralysed, the control rats on the same level of marmite, but without concentrate, remained smooth coated and active, although growth at the time had become almost stationary at a higher level. By the administration of vitamin B (wheat-germ extract, later replaced by marmite) to two of the rats receiving concentrate, death was averted and rapid growth immediately restored. Similarly in Exp. 2, Fig. 2, two animals receiving the very high level of 2.5 % of concentrate declined and died, whereas

¹ Two samples of cod-liver oil concentrate were used, kindly supplied by Lever Bros. and by J. Nathan and Co. The first sample was found by biological test to be effective as a source of vitamin A at the low dosage of 0.01 mg. per rat daily. From colorimetric data it is to be expected that the second sample was even richer by about 50 % in vitamin A. The first sample was reputed to be effective as a source of vitamin D at dosages of about 0.3 mg. daily. Data as to the vitamin D content of the second sample are unfortunately not available, but it is to be suspected that the ratio between vitamin A and vitamin D contents must have been of a similar order.

² It must not be assumed, however, that the concentrate, if administered at higher levels, might not have accelerated the decline. Larger amounts were not given in these experiments, but it may be recalled that in earlier work [Harris and Moore, 1928] we observed a greatly accelerated decline when large excess of concentrate was added to diets totally deficient in vitamin B.

a control animal receiving only 0.02 % of concentrate remained well and grew rapidly. At 0.5 % of concentrate (Exp. 1, Fig. 1) death occurred in the case of only one animal, and at the 0.1 % level there was only a graded retardation in growth rate (Exp. 1, Fig. 1).

Fig. 1. Increasing doses of concentrate are represented by an increasingly solid line for the growth curve.

A given improvement in the growth rate could be effected by means either of a relatively small increase in marmite (vitamin B) keeping vitamins A and D constant or, alternatively, by a much larger decrease in the excess of concentrate (containing vitamins A and D) keeping the marmite (vitamin B) constant. Piebald rats were used in this experiment.

Fig. 2. A confirmatory experiment with piebald rats, but testing a very much higher dose of concentrate, and at two extreme levels only of vitamin B feeding. $\times$ = died.

..... = 0.02 % of concentrate.

— = 2.5 % of concentrate.

8 % *Marmite*. At this level of vitamin B (considered sufficient to permit optimal growth on ordinary diets) the addition of concentrate (0.02 %, 0.1 %, 0.5 %, 1 %, 2 %, 4 %, 8 %, 16 %, 32 %) had no effect on growth rate.

0.5 %) no longer caused death in any instance, but the growth rates of all animals were influenced inversely according to the level at which the concentrate was administered. Thus at the higher levels of concentrate this amount of marmite was now no longer sufficient for optimal growth.

16–32 % *Marmite*. At these levels of vitamin B (considered superfluously high for the promotion of growth on normal diets) the addition of excess of concentrate (0.02 %, 0.1 %, 0.5 %, 2.5 %) could be tolerated without serious retardation in growth rate. Nevertheless two animals receiving maximal doses, 0.5 % and 2.5 % respectively, died suddenly without preliminary decline.

Fig. 3. An experiment with albino and piebald rats, and at a critical "border-line" allowance of marmite. At a high level of vitamin B—right-hand side—moderate dosage with concentrate (vitamins A and D) was apparently harmless; but at the low level of marmite just adequate for maintenance but not growth—left-hand side—the same dosage of concentrate led on the contrary to death with symptoms parallel with those seen in ordinary avitaminosis B. x = animal died. * = animal dying, and killed or put on curative (increased vitamin B) diet (see Fig. 4).

..... = Low A and D (1 drop cod-liver oil per day $\equiv$ 0.001 % concentrate).
 — — = High A and D (0.1 % concentrate).

Thus we have a picture of a gradually increased toleration for the concentrate with each increased marmite allowance. While 0.1 % of concentrate caused inevitable decline at 3.2 % level of marmite, 0.5 % and even 2.5 % (in one case) could be tolerated without apparent ill-effects at 32 % level of marmite.

All the effects so far observed in animals dying through the addition of

excess of vitamins A and D to diets on the border-line with respect to vitamin B have been consistent with simple vitamin B₁ deficiency. Loss of weight, failing appetite, marasmus, and sometimes, eventually, difficulty in use of limbs, and paralysis were the main symptoms. (The picture recalls also that described by Takahashi *et al.* [1925] for biosterin poisoning.) At autopsy there was a picture of inanition, with no macroscopic lesions or abnormalities in the organs apart from a certain diminution in the size of the spleen and thymus which is normally seen in animals which have been depleted of the vitamin B complex.

The high amount of vitamin D in the concentrate was presumably still insufficiently excessive to cause hypervitaminosis D, of which no gross evidence was observed: histological work is still in progress by our colleague, Mr J. R. M. Innes.

Growth curves. The thickness of the black and white lines respectively represents proportional amounts of fat-soluble vitamins and of vitamin B present in the diets so that it is possible to see which diets are overloaded by a relative excess of the former.

—— = cod-liver oil concentrate, 0.1 % of diet.

— = cod-liver oil, 1 drop per animal per day.

—— = marmite, 12.8 % of diet; or 0.2 cc. wheat-germ extract, or 0.75 cc. marmite per animal per day.

—— = marmite, 3.2 to 4 % of diet.

Fig. 4. *Curative experiments* (growth curves, compounded from Figs. 2 and 3). The extra vitamins A + D which proved fatal when added to a low vitamin B ration (animal No. 1), were no longer harmful if balanced by extra vitamin B also (animal No. 2). Similarly animals dying on the "unbalanced," high vitamins A + D—low vitamin B, ration (animal No. 3) could be cured by increasing the vitamin B allowance (animal No. 4). Alternatively removal of excess of vitamins A + D with no increase of vitamin B served to restore the balance.

At A 0.2 cc. of wheat-germ extract was given per day.

At B 0.75 cc. of marmite extract was substituted.

Such large doses of concentrate as we have used in this work may be presumed to have had an adverse effect on reproductive processes, as occurred when cod-liver oil was administered at the same or lower vitamin levels [Harris and Moore, 1928], but this question is still under investigation.

DISCUSSION.

Two different interpretations of the results described might be advanced. The first would be that the high doses of concentrate used are in any case poisonous and that the ill-effects merely become more obvious when the animal is in addition suffering from a partial vitamin B starvation. This interpretation is naturally difficult to disprove, but the very wide variations seen in the degree of toxicity of the concentrate at different levels of marmite afford some evidence to the contrary. It is easy to conceive that differences in the animal's condition might account for varying resistance to the toxic action of concentrate when the amount given was approaching the minimal lethal dose, but if we assume from Exp. 2 that this was about 9 mg. per day, it is difficult to understand how doses as great as 50 mg. and even 250 mg. (in one case) were tolerated in other experiments. Moreover allowances of marmite well above that normally necessary for optimal growth have been shown still to produce marked improvement in the growth rates of rats receiving large excess of concentrate. Such action is difficult to reconcile with the view that the beneficial action of the marmite is exerted only by its general influence on the condition of the animal, since in that case the addition of marmite above the sufficient amount would be superfluous. It will be recalled, however, that the amount of the vitamin B complex necessary to maintain the rat in growth and apparently in perfect health under ordinary conditions will not suffice for the added strain of lactation, as has been shown by Hartwell and others, or for growth on diets rich in protein [Hassan and Drummond, 1927]. Whether or not we assume a definite antagonism between concentrate and the vitamin B complex it would appear that the disposal of excess of concentrate raises a similar demand for increased supplies of vitamin B.

Is one justified then in regarding these effects, produced by adding large amounts of vitamins A and D to a diet low in vitamin B, as an avitaminosis B? Judged by the criteria hitherto adopted, the condition which we have observed would doubtless have been so described. For we have a condition which can be cured or prevented by the addition to the diet of very small amounts of the usual sources of vitamin B (or B₁), viz. 0.2 cc. of wheat-germ extract, or 0.75 cc. of marmite extract—amounts which are no more than the optimal protective doses. Next we find the symptoms which supervene, as well as the *post-mortem* findings, to be consistent (at any rate in general outline) with those observed in vitamin B (or B₁) deficiency in the rat. Finally, at all of the levels of concentrate dosages employed in this work ill-effects were rarely

to be seen whenever the marmite allowance was increased to a sufficient extent. Therefore the concentrate would not appear to be toxic *per se* but only when accompanied by a relatively inadequate vitamin B allowance.

That the effects which were produced by the vitamin A and D concentrate may have been caused by some substances other than either of the two vitamins themselves is not inconceivable¹. But if so, these substances must be present in amounts so small as to bring them in their activity into the same category as the vitamins. For the amount of concentrate which served to unbalance the "low B" diet, and to make the difference between maintenance or rapid loss of weight and death was of the order of 9 mg. per rat *per diem*.

At the same time the relatively large increase above the minimal dose in the amount of the fat-soluble vitamin preparation which is needed to convert the partial vitamin B deficiency into an acute deficiency is noteworthy. Thus in the experiment shown in Fig. 3 we were working with a dosage of concentrate equivalent to 1000 times the minimal dose of vitamin A, or 30 times the minimal dose of vitamin D, in order to convert a bare maintenance diet into an undoubtedly fatal one. Although no attempt to give a strictly mathematical expression to our results is intended, a scrutiny of the results in Fig. 1 shows that at the particular vitamin levels there obtaining a fivefold increase, at least, in the fat-soluble concentrate was needed to cause the same lowering in growth rate as could be obtained by halving the vitamin B allowance.

While on the evidence at present available it is impossible definitely to assign the toxic action of concentrate to either vitamin A or vitamin D, it may be pointed out that the latter was administered at levels of only 6 to 750 times the minimal physiological dose, and we have found that doses up to 1000 times the minimal do not give rise to pronounced untoward symptoms when administered under similar conditions in the form of irradiated ergosterol. Interest may therefore first be centred on vitamin A which in these experiments reached the much higher levels of 200 to 25,000 times the minimum dose. As further evidence tending to exclude vitamin D, it is found (L. J. H.) that the severity of hypervitaminosis D (irradiated ergosterol, 0.05 % of diet) is not appreciably lessened when marmite is increased to 5 times, or orange juice to 12 times, the normally adequate level. The substitution of concentrated orange juice for all the carbohydrate (or carbohydrate plus fat) in a normal synthetic diet, however, gave rise to phenomenally rapid growth rates in young rats, viz. up to 5.5 g. per day.

¹ This criticism was indeed raised by Drummond, Channon and Coward [1925] to the original claim of Takahashi *et al.* that "biosterin" possessed toxic properties. The present work suggests the possibility that the contradiction between the evidence of Takahashi and his colleagues and Drummond, Channon and Coward regarding the toxic action of cod-liver oil concentrates may to some degree have been due to variations in vitamin B allowances.

SUMMARY.

1. The need of the rat for marmite (vitamin B complex) is increased *pro rata* when increasing excess of cod-liver oil concentrate (vitamins A and D) is administered concurrently.

2. Thus, in order to produce any given rate of submaximal growth, more and more marmite must be given as more and more of the excess of concentrate accompanies it in the diet.

3. Again, a restricted amount of marmite which is normally adequate for prolonged maintenance becomes inadequate to prevent death when excessive concentrate is given. Rats dying under these conditions could then be cured by administration of additional vitamin B (as wheat-germ extract or marmite) without removal of the extra concentrate.

4. Hence an amount of concentrate which is harmful when given with a moderate marmite allowance is no longer harmful when given with a sufficiently augmented marmite allowance.

5. It is considered most probable that this antagonistic effect is exerted between the vitamin B complex of the marmite, etc., and the vitamin A of the concentrate, or cod-liver oil; but the possible action of other unidentified substances is not excluded.

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