# CLXXXII. "HYPERVITAMINOSIS" AND "VITAMIN BALANCE[1]."

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The experimental observation that vitamins exert their normal physiological action when consumed in very minute doses leads naturally to the enquiry, "Have the vitamins any injurious effect when consumed in abnormally large amounts?" A similar, and probably closely related, question also arises, "Does an increased or diminished consumption of one vitamin affect the body's requirements for the others[2]?" A great number of past writers have assumed, either tacitly or directly, that both these questions could be answered in the negative. In the present communication an attempt is made to collate and extend certain evidence to the contrary.

In regard to the water-soluble vitamins no evidence is as yet available to suggest that any ill-effects result from overdosing, though work with highly concentrated preparations has mainly been limited to the routine testing of minimal effective doses. In the case of the fat-soluble vitamins, on the other hand, several instances of supposed hypervitaminosis have been recorded, and several attempts have also been made to show that the fat-soluble and water-soluble requirements of the animal are to some extent interrelated.

In this country the earliest recorded work bearing on these questions is that of Hopkins [1923], who in the course of the Cameron lecture for 1923 mentioned that he had found that excess of cod-liver oil was often injurious to a rat unless "balanced" by an increased marmite (vitamin B) allowance. Somewhat later, in 1925, the work of Takahashi and his co-workers became generally known. To Takahashi is due the first suggestion that vitamins could have a lethal effect. Between 1922 and 1925 several papers had been published, in Japanese journals [1922, 1923], in which it was claimed that vitamin A (biosterin) had been isolated in a state of purity, and in the English article describing the substance [1925] it was reported that while daily doses of 0·004 mg. sufficed for the normal growth of rats, doses of 1·6 mg. invariably

[1] Certain conclusions here given were briefly summarised in the *Lancet* (1928), ii. 892.

[2] A particularised form of this question was put by Funk [1922]. "If, for example, the quantity of vitamine A is too little the requirements for vitamine B are perhaps increased. However, there are as yet no available data on this point." Funk, it will be noticed, is here suggesting the exact reverse of a balance, as understood in this paper.

caused failure in growth and death. Drummond and his collaborators [1925] however had been engaged in a patient research in the same field, and were able to contradict Takahashi's claim to have isolated the vitamin. Using carefully prepared concentrates no toxic action could be noticed in large doses, and it was suggested that the Japanese worker had been deceived by the presence in his preparation of poisonous by-products. Other investigators (*e.g.* Magliano [1925]) likewise stated that large excess of vitamin A or B, or of both, was harmless to a growing animal. In the face of such opposition interest in the problem naturally became small, and the possibility of vitamin excess, or vitamin balance, was generally held to be excluded.

From time to time, however, various isolated and little known observations have been recorded, mostly in somewhat inaccessible literature, which possibly have not received the attention they deserve. Mouriquand and Michel [1922], also Bezssonoff [1923], had spoken of the noxious effect of large doses of cod-liver oil in scurvy, and on somewhat similar lines Euler and Widell [1925] made the surprising claim that excess of cod-liver oil caused disturbances in the growth and bone formation of rats unless balanced by vitamin C[1], a factor which is generally supposed to be unnecessary for the well-being of that animal. More recently Agduhr [1926] has reported degenerative changes in the heart after the feeding of excessive amounts of the oil to various animals, but these results have been attributed by Höjer [1926] mainly to an inadequate provision of the vitamin B complex. Hartwell [1927] and Sure [1927] have also carried out isolated work on the excessive feeding of cod-liver oil, special attention having been drawn to the injurious action of large excess of it during gestation.

In the foregoing work, it will be noted, cod-liver oil or its concentrates were used as sources of vitamin excess, and there was little to indicate whether vitamin A, vitamin D or some other constituent of the oil was responsible for the ill-effects observed. Recently irradiated ergosterol has largely replaced cod-liver oil as a source of vitamin D, and as a result of the investigations of Pfannenstiel [1927] and Kreitmair and Moll [1928] substantial evidence has accumulated to justify at least the suggestion that toxic properties may be attributed to this vitamin *per se*, though there is no reason to suppose that it is necessarily the only toxic factor present in vitamin concentrates. The second-named authors, after a thorough and painstaking research, have reported fatal effects resulting from massive doses in a variety of different species, with notable exceptions in the case of the hen and axolotl. They observed, *post mortem*, extensive depositions of calcium and a generalised sclerosis,—phenomena of much interest in view of the known action of vitamin D in increasing Ca (or P) absorption. That these records should be received not without some scepticism, or at least confusion, is understandable, for

[1] In further development of this line of thought Grant [1926] concludes that for normal development of the teeth it is necessary to have an adequate balance between the dietary calcium, vitamin C and vitamin D, excess of one magnifying the effect of deficiency of the others.

numerous other workers have recorded the apparent harmlessness to a human being of "large" doses of irradiated ergosterol (Kroetz [1927]; see also Havard and Hoyle [1928]) or to a rat up to 10,000 times the minimal effective dose (Rosenheim and Webster [1927]).

During the past year an attempt has been made in our laboratory to collate experimentally some of these scattered observations. A thorough survey is in progress, but we think it advisable to record the results so far obtained, which, in a general way, confirm definitely the idea of a harmful effect resulting from excessive intake of certain materials rich in fat-soluble vitamins.

## Experimental.

All the experimental work recorded hereunder has been done on rats, either albino or piebald. Our procedure differed from that of Kreitmair and Moll in that growing animals were used, and that the vitamin preparations were administered mixed with the ration, instead of separately with forcible feeding when necessary or by intravenous injection.

### I. *Excessive irradiated ergosterol.*

*Technique.* Young piebald rats, generally 40–50 g. in weight, were given "complete synthetic diets" containing irradiated ergosterol, in the concentrations 0·00001, 0·001 and 0·1 %. Animals from a single litter were distributed as evenly as possible. For controls, litter mates were given the same synthetic diet, but containing non-irradiated ergosterol and heated ergosterol in the same concentrations, and no ergosterol. Each rat in this experiment was isolated in a separate all-metal cage having a netted floor of somewhat coarse mesh.

*Diets.* The diet consisted of:

200 g. purified caseinogen,
600 g. rice starch,
50 g. salt mixture,
190 cc. arachis oil;

the last ingredient containing the appropriate quantity of irradiated, non-irradiated, or heated ergosterol as the case might be. The irradiated ergosterol was supplied to us by Messrs British Drug Houses[1]. The heated ergosterol was prepared by leaving non-irradiated ergosterol in an open dish for 3 hours at a temperature slightly above the melting point. Each animal received in

[1] Messrs British Drug Houses have provided us with the following details of their procedure. A 0·5 % solution of ergosterol in absolute alcohol was subjected to ultra-violet radiation in a thin layer in the absence of oxygen, except that no attempt was made to remove dissolved oxygen from the alcohol. The duration of the exposure was 15 minutes. The solution was concentrated to a small bulk at a temperature not exceeding 40° with the use of a vacuum with a small stream of nitrogen. The concentrated solution was cooled and allowed to stand, for unchanged ergosterol to crystallise out. 14·9 % of the ergosterol originally taken was removed unchanged. The solution was further concentrated as before, transferred to a desiccator and the remainder of the solvent removed *in vacuo* at room temperature. We are informed by Messrs British Drug Houses that a different procedure is employed by them for their commercial preparation of

addition two drops of cod-liver oil daily, and 0·75 cc. of an alcoholic extract of marmite[1] was added daily to each animal's ration.

*Results.* All animals receiving 0·1 % of irradiated ergosterol in their diets lost weight rapidly and were dead after the lapse of 20 days and upwards. All animals receiving the smaller concentrations of irradiated ergosterol however grew normally and appeared to thrive during the same period. Nor could any ill effect be observed during the test period in any of the controls receiving the various concentrations of non-irradiated or of heated ergosterol (see Fig. 1).

The rats receiving the toxic concentration of 0·1 % of irradiated ergosterol were observed to lose their appetite early in the experiment, and this loss gradually increased. Diarrhoea set in generally at about the 5th day. Lesions on the hind paws superficially bearing some resemblance to pellagra, but of no marked severity, frequently attracted attention after a fortnight. The coats became greasy and very rough, and there was marked cachexia. The *post mortem* appearance was that of inanition, recalling therefore an effect of vitamin B deprivation. The pathological changes are still under examination and will form the subject of a later communication.

In Fig. 2 are shown the results of an experiment in which the above observations were repeated, using diets in which the ergosterol, irradiated and non-irradiated, had been incorporated three months previously; the daily food consumption was also noted approximately.

It is obvious, of course, that the ill-effects may quite conceivably have been caused by impurities in the irradiated ergosterol and not by the vitamin D itself, but since we have found that heated ergosterol has not the same deleterious effect it is clear that impurities which are common to the processes of heating and irradiation cannot be held responsible. Similar tests are now in progress on specimens of activated ergosterol in which the D factor has been destroyed by over-exposure to irradiation[2]. Among other aspects of the problem, we are investigating at present the following: intestinal reaction; the Ca and P absorption and blood levels; electro-cardiographic records.

### II. *Excessive irradiated ergosterol with increased B (and C) vitamin allowance.*

Efforts have been directed to determine whether the harmful effects of excess of irradiated ergosterol could be "balanced" by an increase of the vitamin B-complex. In one experiment, where the marmite allowance was

irradiated ergosterol ("radiostol"). The possibility may be borne in mind of different methods of irradiation producing a different degree of toxicity in any by-products, apart from the clear evidence of true hypervitaminosis-D as revealed in excessive calcification, hypercalcaemia, hyperphosphaemia (Kreitmair and Moll, Hess and Lewis; see Discussion, p. 1475).

[1] The extract is that used at the Cambridge Biochemical Laboratory: marmite is shaken with successive quantities of 85 % alcohol, the alcohol is removed by evaporation from the extract thus obtained, and the resulting fluid is filtered and made up with distilled water to a total volume equal to the weight of the marmite originally taken.

[2] We have found that a lethal dose of irradiated ergosterol becomes non-toxic concurrently with the destruction of vitamin D by over-irradiation.

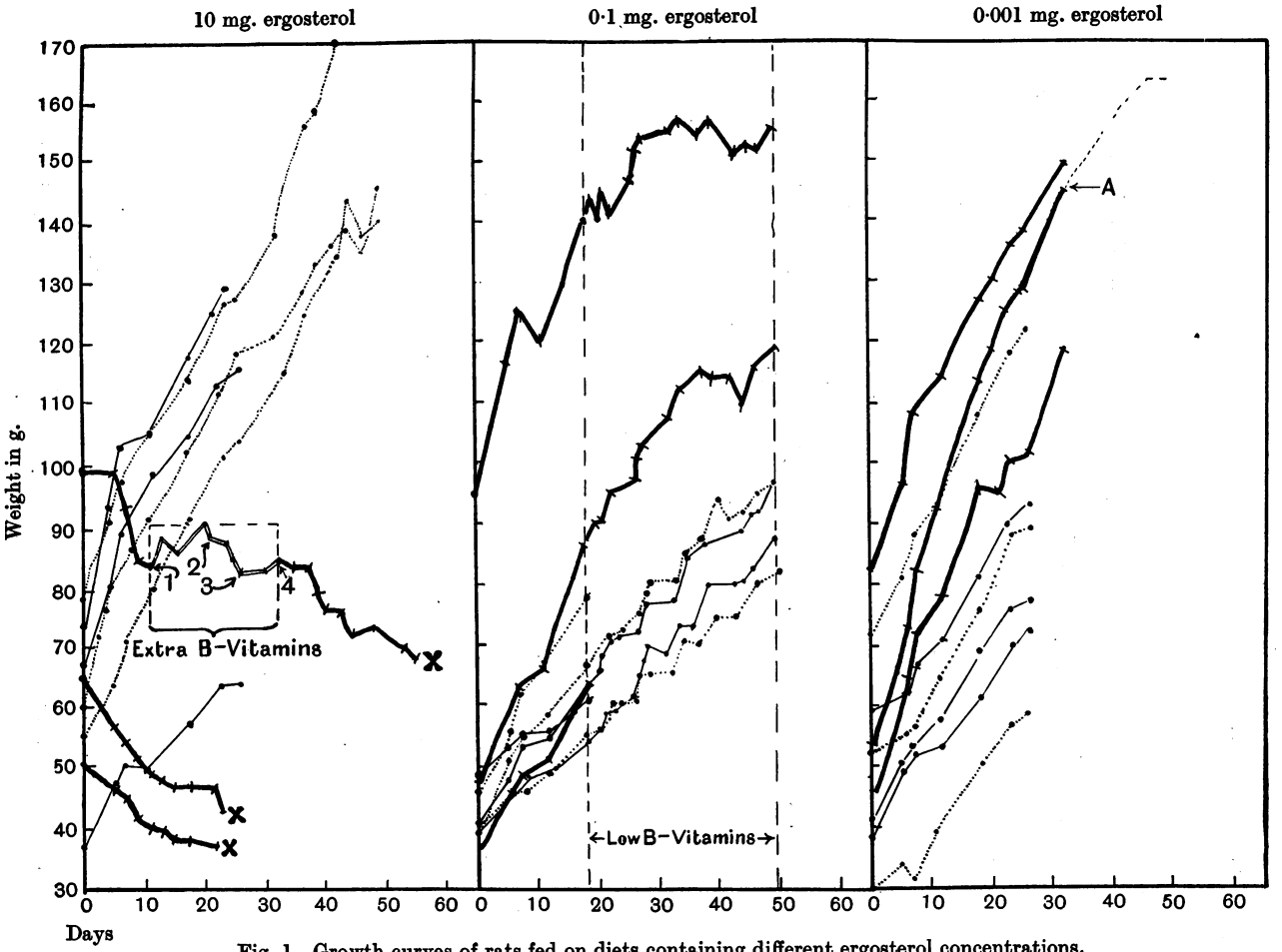


Fig. 1. Growth curves of rats fed on diets containing different ergosterol concentrations.

Rapid loss of weight and death occurred with 10 mg. of irradiated ergosterol per 10 g. of diet. At the lower concentrations of 0·1 mg. and 0·001 mg. of irradiated ergosterol per 10 g. of diet, growth occurred over the same period. With non-irradiated and with heated ergosterol growth was supported at all three levels.

(At the point "1" the marmite allowance was increased from 0·75 to 3·0 cc., at "2" 0·5 cc. of a wheat germ extract, and at "3" 10 cc. of orange juice were added, per diem. At "4" these additions were discontinued.)

Irradiated ergosterol.

Non-irradiated ergosterol.

Irradiated ergosterol, extra B vitamins

Heated ergosterol.

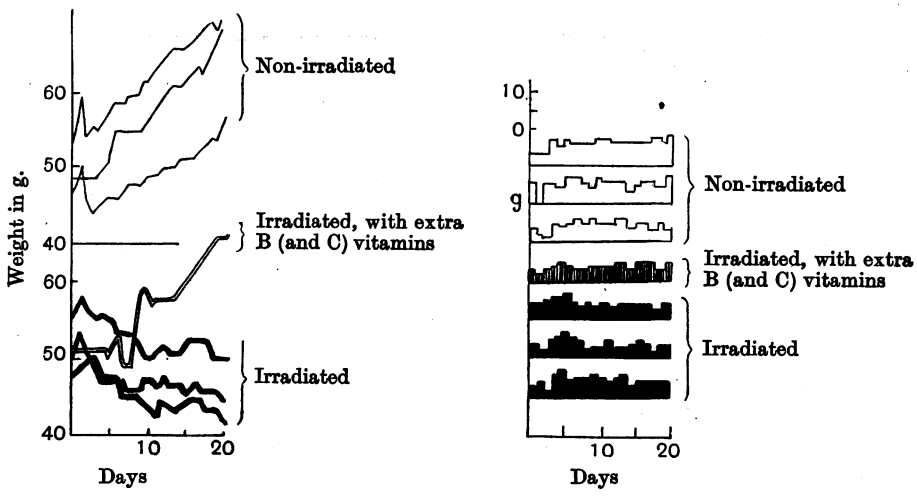


Fig. 2. Growth curves and food intakes. Diet contained 0·1 % ergosterol, incorporated 3 months previously.

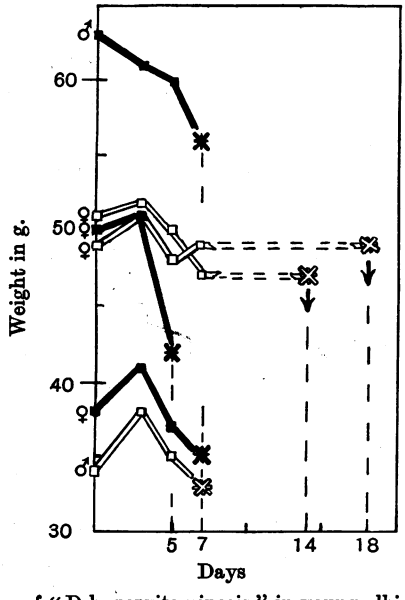


Fig. 3. Comparison of "D-hypervitaminosis," in young albino rats, on normal and fourfold vitamin B allowance.

(0·1 % irradiated ergosterol.)

0·75 cc. marmite extract.

3·00 cc. marmite extract.

Denote date of death.

increased to only four times the normal minimal level for good growth (viz. from 0·75 cc. of our extract to 3 cc.), a negative result was obtained. The rats (albinos in this instance) declined almost as rapidly and died about as soon as those whose marmite allowance remained at one-quarter the amount (Fig. 3). (An experimental difficulty is that rats on a normal diet will not thrive when given more than 3–4 cc. of our marmite extract, so that while the vitamin D has been increased 100,000 times above the minimal dose the vitamin B in the present case has not been increased by more than four times the minimum.)

In the opposite direction we can at present refer to two animals only, which when given still more vitamin B (fourfold marmite, plus wheat germ extract and orange juice) remained steady or gained in weight (see Figs. 1 and 2) while their mates, whose ration was not so balanced, declined rapidly, as mentioned above. Appetite improved, the animals remained apparently healthy, and their coats continued in good condition instead of being rough and greasy. Diarrhoea, however, was not averted. One animal on having the vitamin B allowance reduced to the normal level then began to lose weight and later died (see Fig. 1).

### III. *The apparent ability of the rat to discriminate between normal doses and toxic overdoses.*

An attempt was made to produce severe "hypervitaminosis" in rats by the administration of a single massive dose of vitamin D. Two rats were offered 5 g. of synthetic diet containing 0·25 g. of irradiated ergosterol but they refused it, consuming only a negligible quantity, while a litter mate readily consumed 5 g. of the same diet differing only in containing 0·25 g. of ergosterol in the non-irradiated form. The former animals lost weight rapidly from starvation and the experiment had to be abandoned; the latter grew normally for 18 days following (*A* in Fig. 1) and showed no ill effects, when the observations were concluded.

In a confirmatory experiment two rats were taken off a mixed dietary and offered the choice between synthetic diets containing excessive (1) non-irradiated, and (2) irradiated ergosterol, and also (3) no ergosterol. (The third named was only added after the lapse of a day when little of the first two had yet been eaten.) The ergosterol (0·025 g.) was added in chloroform solution to 5 g. of diet and the chloroform evaporated, the diet with no ergosterol being treated with chloroform in the same way. Allowance was made for the small amount of water lost from the ration during the time it was offered. As the figures below show, the non-irradiated ergosterol was eaten in preference to the irradiated. The experiment was repeated a second time with similar results.

| | Food eaten (g.) | | | |
|---|---|---|---|---|
| | Rat No. 1 | Rat No. 1. Repeat | Rat No. 2 | Rat No. 2. Repeat |
| Containing irradiated ergosterol | 0 | 0 | 0·3 | 0 |
| Containing non-irradiated ergosterol | 3·0 | 4·0 (all gone) | 5·2 | 2·0 (all gone) |
| Containing no ergosterol | 4·3 | None offered | 1·0 | None offered |

As in Section I, we have no evidence whether the effect be due specifically to the high concentration of vitamin or to some distasteful impurity. Considerations of expense almost preclude any large-scale repetition of the present observation, but it may be concluded that it is important to consider the question of loss of appetite or distaste for food in interpreting the results in the other sections[1].

IV. *Vitamin B deprivation accompanied by increased vitamin D allowance.*

These experiments were planned in order to determine if one could discover any very simple relationship of "vitamin balance" in the sense that excess of one vitamin might be the more harmful in proportion to coexisting deficiency of the other, and *vice versa*.

*Procedure.* A concentration of 0·001 % of irradiated ergosterol was employed, *i.e.* one-hundredth of what may be called the lethal concentration (or one-hundredth of Kreitmair and Moll's toxic dose of 10 mg., assuming the animal to consume, as it does, approximately 10 g. of food). As a preliminary diet for 18 days, three rats received the complete ration containing 0·75 cc. of the marmite extract already described and 0·001 % of ergosterol, non-irradiated, irradiated and heated in the three cases. During this period somewhat subnormal growth occurred. Two rats in each of the three sets then received the diminished allowance of only 0·25 cc. of marmite extract, and one rat in each set no vitamin B.

*Result.* In the three animals deprived of all vitamin B, cessation of growth, loss of weight and finally death took place at much the same rate or time, irrespective of the amount of vitamin D in the diet (see Fig. 4). Rats receiving the restricted (one-third adequate) amount of vitamin B represented by 0·25 cc. of marmite extract showed no striking difference in their growth curves with variation in their vitamin D allowance (see Fig. 1, centre).

The rats receiving about 1000 times the adequate supply of vitamin D, it will be observed, succumbed no more rapidly to B-avitaminosis than is usual with rats receiving no increased vitamin D. Nor was one-hundredth of a toxic dose of irradiated ergosterol converted into a toxic dose when the vitamin B allowance was reduced to one-third. The experiment is of course not necessarily conclusive against the existence of a "vitamin balance", except in the special sense indicated.

IV. *Excessive cod-liver oil and retarded growth.*

Two carefully matched sets (*A* and *B*) of young albino rats were used, each set being divided into four groups. Similar synthetic diets (20 % caseinogen, 15 % fat, 40 % starch, 5 % salts) were fed to each set, but whereas set *A* had the 15 % fat supplied by a highly potent cod-liver oil, set *B* received the

[1] It may be added that these observations can be paralleled in another investigation, in the course of which one of us (L. J. H.) has found that an animal starved of one of the B vitamins is able to discriminate between diets containing a bare sufficiency and those devoid of it.

corresponding amount of inactive arachis oil together with a single drop of cod-liver oil per rat per day, to ensure a bare sufficiency of vitamins A and D. The groups in each set were then placed on graded allowances (1·6 %, 3·2 %, 6·4 %, 12·8 %) of our standard marmite extract, in order that the effect of the two oils might be compared at various levels of vitamin B intake.

*Results.* Invariably rats in set *A* (15 % cod-liver oil) recorded lower growth rates than carefully matched litter mates in set *B* (15 % arachis oil) (see Fig. 5). In Fig. 7, for convenience in presentation, the curves for each

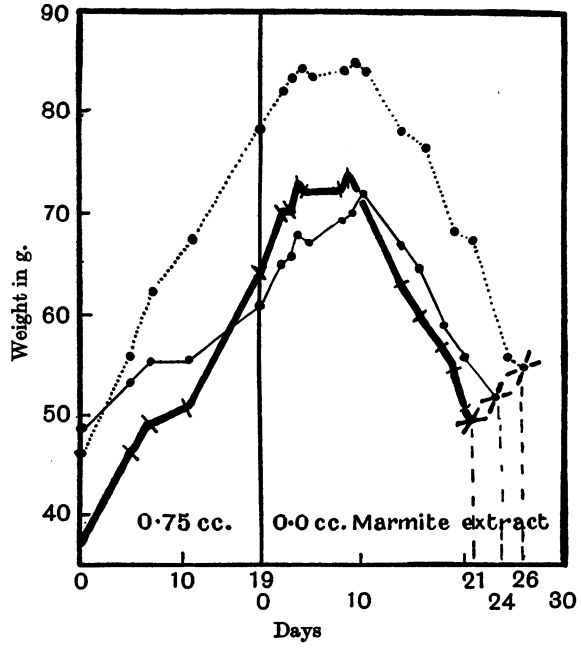


Fig. 4. B-avitaminosis with increased vitamin D allowance.
The extra vitamin D provided did not appreciably retard the avitaminosis.

——— Irradiated ergosterol, 0·1 mg. per 10 g. diet.
..... Non-irradiated „ „ „
——— Heated „ „ „

group have been compounded and averaged. Differences were most marked at the intermediate marmite concentrations (3·2 and 6·4 %) adequate for maintenance but inadequate for rapid growth. Only small differences were observed at the highest (12·8 %) and lowest (1·6 %) marmite levels, the latter being inadequate for prolonged maintenance. The animals receiving cod-liver oil, except for marked abnormalities discussed under the next heading, remained in fair condition, though they could readily be distinguished from animals of the other groups by the roughness of their coats, which became distinctly yellowish in colour. The area around the genitals and anus was also found invariably to be stained a deep yellowish colour.

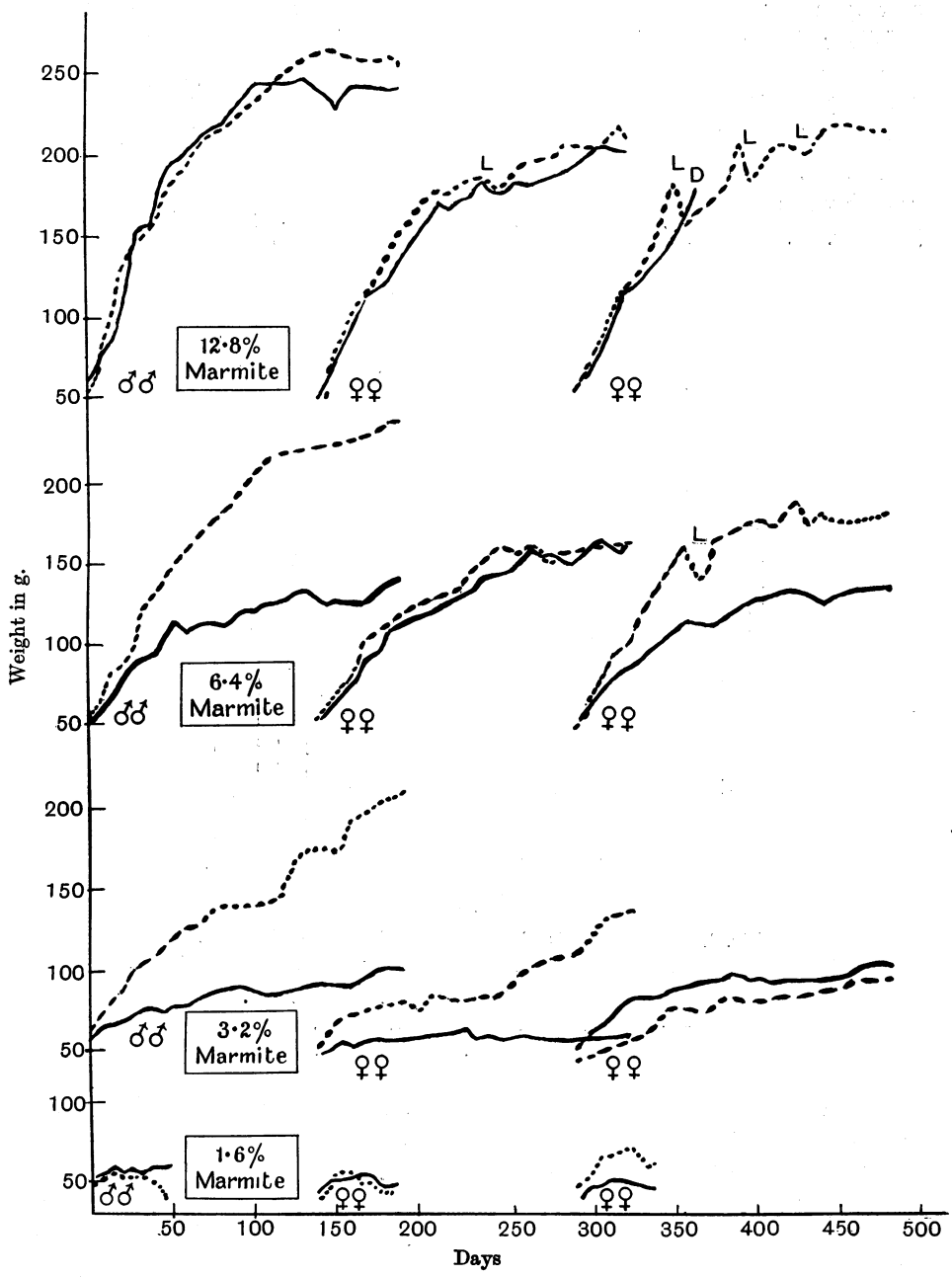


Fig. 5. Excessive cod-liver oil.
Continuous curves—Cod-liver oil diet.
Dotted curves—Arachis oil diet.

To confirm the above results the experiment was repeated at those concentrations of marmite (3·2 and 6·4 %) at which the greatest divergence had been observed. Exactly similar results were obtained (see Fig. 6).

V. *Failure of pregnancy on excessive cod-liver oil.*

In the preceding experiments sexes had remained together for several months, and as a result substantial support is afforded to the view that excess of cod-liver oil leads to failure of reproduction in rats. Since a detailed investigation was not made it is impossible to state whether failure in conception or resorption was usually the cause of sterility. The litters obtained were as follows.

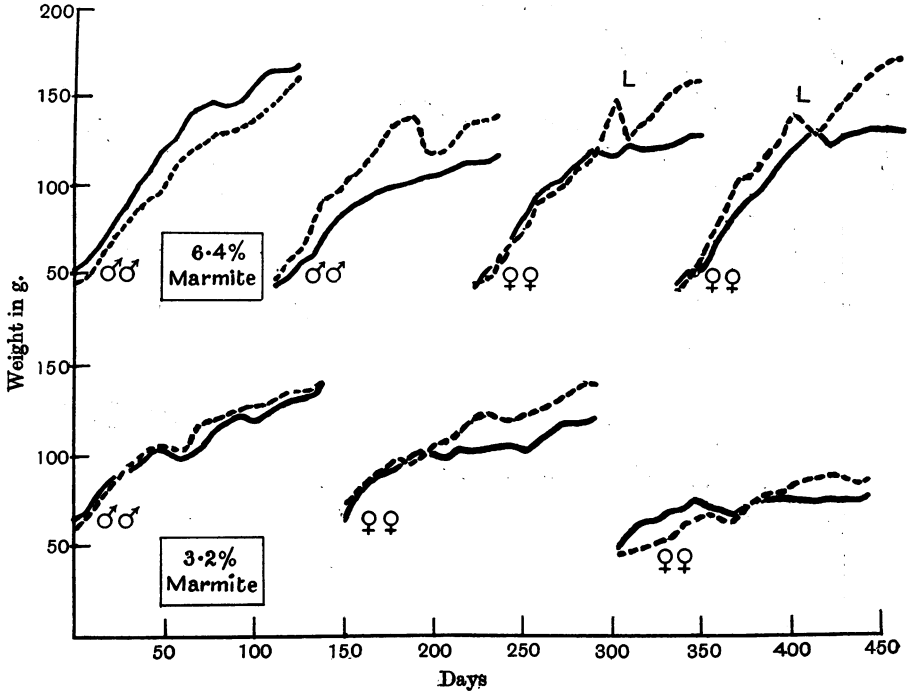


Fig. 6. Excessive cod-liver oil.
Continuous curves—Cod-liver oil diet.
Dotted curves—Arachis oil diet.

*Set A.* No litters were obtained in any group. One rat in the group receiving 12·8 % marmite became pregnant, but died during pregnancy. An autopsy revealed severe haemorrhage into the uterus. One developed foetus was found together with several small, apparently degenerated foetuses.

*Set B.* Seven litters were obtained in all, four from two rats receiving 12·8 % of marmite extract in their diet, three from four rats receiving 6·4 %. No litters were obtained from the groups receiving 3·2 % and 1·6 % of marmite extract. In no case were litters successfully reared, but they were often kept for periods up to 20 days.

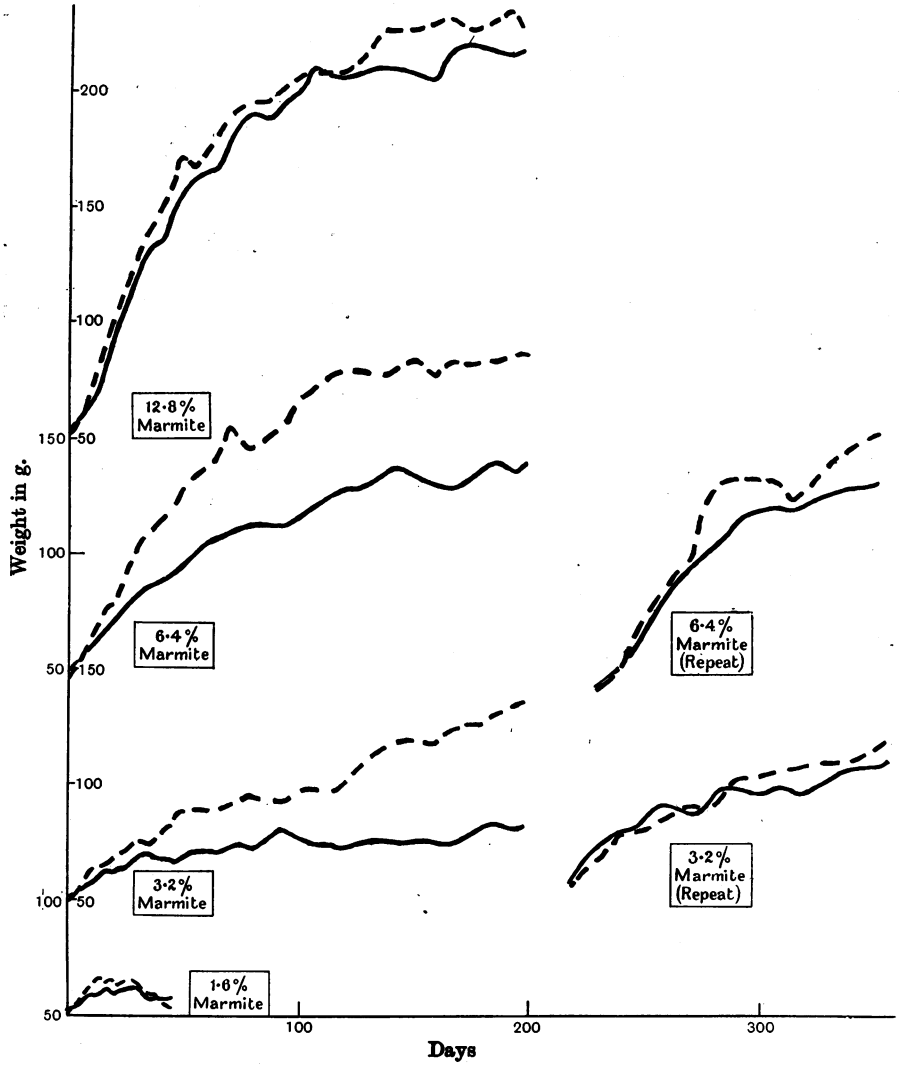


Fig. 7. Excessive cod-liver oil.

Average growth rates of groups.
Continuous curves—Cod-liver oil diet.
Dotted curves—Arachis oil diet.

VI. *Excess of cod-liver oil concentrate with deprivation of B vitamins.*

Two groups (*A* and *B*) of three young piebald rats each were used. Each received a standard basal diet (B.D.H.) without addition of marmite. For each rat in group *A* five drops of the unsaponifiable matter of cod-liver oil[1] were added, which were carefully stirred into the diet.

[1] Obtained through the courtesy of Messrs Lever Bros.

Both groups, through deprivation of the vitamin B complex, soon began to lose weight (Fig. 8), but in group *A* (receiving concentrate) such loss was much more rapid than in group *B*. After a few days a marked difference in appearance between the two groups could be observed. The rats in group *B*, though gradually weakening, were no more rough coated than was consistent with a short period of vitamin B deprivation. The rats in group *A*, on the other hand, began to lose hair around the mouth and paws, and skin lesions and bleeding soon developed. The condition superficially resembled severe pellagra[1] or the "egg-white" condition reported by Boas [1927].

In preliminary experiments attempts to alleviate the condition by the administration of marmite met with failure. Further experiments in this connection are now in progress.

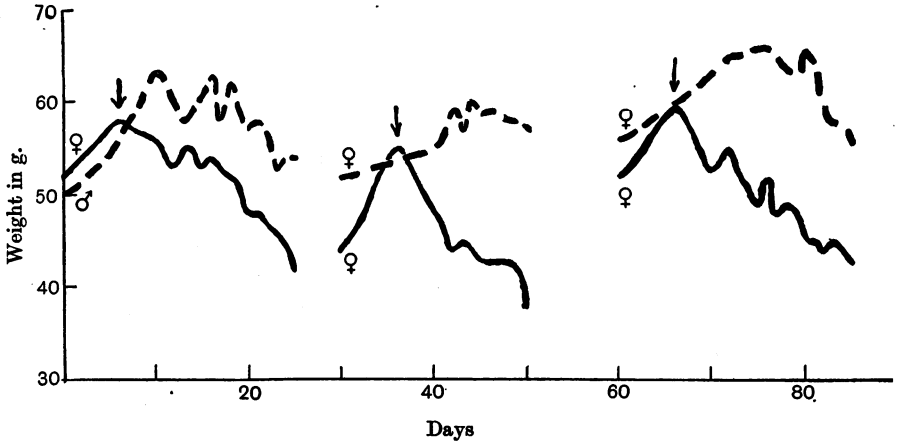


Fig. 8. Excess of cod-liver oil concentrate on vitamin B-free diet.
Continuous curves—With concentrate.
Dotted curves—Without concentrate.
↓ = Concentrate first administered.

## Discussion.

"*Hypervitaminosis.*" In support of the contention of Pfannenstiel and Kreitmair and Moll that vitamin D is injurious when consumed in large excess we have found that irradiated ergosterol, cod-liver oil and cod-liver oil concentrate are all more or less harmful in excess, the degree of toxicity in each case being roughly in line with the amount of vitamin D reputed to be present. Moreover, crystalline ergosterol, over-irradiated ergosterol and ergosterol resinised by heating have been found apparently harmless when consumed for the same periods at levels definitely lethal in the case of irra-

[1] Symptoms of vitamin $B_2$ deficiency are, of course, not observed in simple deprivation of the vitamin B complex, because the animal succumbs to the deficiency of vitamin $B_1$ before the effect of $B_2$ deficiency becomes apparent.

diated ergosterol—which suggests a close parallelism between the development of antirachitic and of toxic properties.

As a possible criticism it might be argued that the injurious effects could in all cases be attributed not to the vitamin but to other substances accompanying it which might have been either directly poisonous or else indirectly harmful by rendering the diets too distasteful to be eaten. To the question of direct poisoning no complete answer can be given, since, for the present, vitamin D cannot be administered free from impurities. To the question of indirect harm by starvation the answer may be given that the rats, although suffering considerable diminution in appetite on the introduction of the irradiated ergosterol or cod-liver oil concentrate into the diet, at least consumed sufficient amounts of the food to keep them alive for periods of about 20 days. Moreover, the possibility cannot yet be excluded of excess of vitamin D serving to unbalance the vitamin B allowance, and thereby rendering insufficient what would be normally an adequacy. In this case the most noticeable effect of any such virtual deficiency of vitamin B would indeed be a loss of appetite such as is observed. Again, rats receiving excess of irradiated ergosterol developed various symptoms, including diarrhoea, which could not be attributed to simple starvation.

Although we consider our results to have been consistent with the theory of hypervitaminosis it is not desired for the present to claim for them a too liberal interpretation. All that can be stated with certainty is that several materials commonly used as rich sources of vitamins (A and D, or D only) have been found to exert definitely harmful effects when administered to rats in amounts greatly in excess of the sufficient physiological level. Although a weight of evidence points to the toxic action of excess of vitamin D there is no necessity to assume that the ill effects observed were invariably attributable to that factor. Granted the toxicity of vitamin D excess, the question of vitamin A excess, for example, remains open. In cod-liver oil there are certainly many constituents of whose chemical nature and properties little is known. We allude to this because the simpler view is sometimes adopted that replacement of inert fats by cod-liver oil in a diet can be represented from the nutritional point of view simply as an increase in the A and D factors.

To obtain further evidence as to the degree of association of the toxic effects with vitamin D, *per se*, experiments are in progress to decide whether materials rich in the vitamin retain or lose such effects when the vitamin is destroyed, *e.g.* by prolonged over-irradiation. Further light is also being thrown upon this issue by investigations now proceeding on the effect of massive vitamin D intake upon calcium and phosphorus metabolism and on acid-base equilibrium in the gut.

"*Vitamin balance.*" Since rats receiving only the normal allowance of marmite (B vitamins) suffered from no marked symptoms of vitamin B deficiency when irradiated ergosterol was given at a rate 1000 times the minimal physiological

dose, it must be obvious that any possibility of simple arithmetical equivalence in balance between vitamin D and the B vitamins is out of the question. Pointing to the same conclusion we have the failure of this concentration of irradiated ergosterol to accelerate the decline which results from deprivation of the B vitamins (in which case the balance would be upset to the utmost degree).

Evidence as to the existence of a less quantitative form of balance, *e.g.* the ability of a relatively slightly increased vitamin B intake to modify the harmful effect of large excess of irradiated ergosterol is less clear-cut. Rats receiving "toxic" amounts of irradiated ergosterol (*i.e.* 100,000 × minimal effective dose) in a limited number of cases seemed to derive but slight benefit from a fourfold increase in the marmite allowance, but by the further introduction of wheat-germ extract and orange juice into the diet some favourable results have been obtained. These observations of course need extending.

In regard to the retardation of growth by unconcentrated cod-liver oil at various levels of marmite allowance, a study of the growth curves (Figs. 5, 6 and 7) might be considered to afford evidence of a balance between vitamins A (and D) and B—since in the presence of cod-liver oil larger amounts of marmite were required to produce any given growth rate than in its absence. It is not however our desire to advance this view, since the apparent balance can be more simply explained as a superimposition of the ill effects of large excess of cod-liver oil upon those incidental to partial vitamin B deprivation. Similarly, the fact, already alluded to, that certain of the symptoms of "D hypervitaminosis" were superficially similar to those of vitamin B deficiency may be no more than accidental.

Summarising the evidence we would suggest that, while the conception of a strictly quantitative balance cannot be tenable in any general sense, the possibility of large excess of one vitamin emphasising the effects of deficiency of another cannot as yet be ruled out.

*Practical applications.* In view of possible alarmist fears prejudicial to the use of the preparations (irradiated ergosterol and cod-liver oil concentrate) placed at our disposal by manufacturing houses we wish in conclusion to make clear that the doses required to produce toxic effects were at levels altogether outside those that are in practice used commercially or that could be employed in clinical practice.

In the first place, we have ourselves tested certain samples of margarine in which the vitamins A and D concentrate is employed and have satisfied ourselves both as to its vitamin value and the absence of any ill effects.

As to the clinical aspect[1], roughly calculating our doses for a child of 28 pounds weight on a basis of relative body weight, we find that 2 g. of irradiated ergosterol, 20 g. of cod-liver oil concentrate and 300 g. of cod-liver oil would

[1] See Harris [1928].

have been administered daily. Toxic effects at such enormous dosages should not in any way discourage the rational use of the properly standardised materials[1] at the ascertainable correct physiological levels[2].

SUMMARY.

(1) Young rats lost weight rapidly and died when receiving synthetic diets containing 0·1 % of an irradiated (but not non-irradiated, over-irradiated or heated) ergosterol (*i.e.* about 100,000 times a minimal protective dose). There was loss of appetite, ill condition of coats, etc., diarrhoea, and inanition.

(2) When the vitamin B (marmite) allowance was increased to only four times the normally adequate level no appreciable alleviation of these symptoms resulted. In the two cases where still further vitamin B (and C) (wheat-germ extract plus orange juice) was administered, loss of weight was prevented.

(3) Results due to "toxicity" are contrasted with those due to mere loss of appetite. Reference is made to the apparent ability of the rat to discriminate in its choice of diets. In a quantitative study rats were found to refuse food overloaded with irradiated ergosterol (5 %).

(4) Rats were found to have lower growth rates compared with litter mates, and to have rough coats, when cod-liver oil was substituted for 15 % of arachis oil (inactive) in a ration containing restricted allowances of vitamin B complex.

(5) In confirmation of Hartwell and of Sure, we observed that normal gestation always failed in rats receiving a diet containing 15 % of cod-liver oil.

(6) Rats receiving massive doses of vitamins A and D concentrate from cod-liver oil, in conjunction with a diet deficient in the vitamin B complex, developed loss of hair and severe skin lesions.

The feeding and care of the experimental animals have been in the experienced hands of Mr Alfred Ward.

[1] Different specimens of irradiated ergosterol, for example, probably vary considerably in vitamin D content.

[2] The report has recently appeared of Hess and Lewis [1928], who as a routine treated their rachitic patients with irradiated ergosterol at the admittedly high level of 2·5–5 mg. *per diem*, equivalent to about 35–70 teaspoonfuls of cod-liver oil. (This high dosage, however, has been widely advocated and appears to have some vogue.) Remarkable benefit resulted in every one of their large number of cases; there were, however, several instances of "eburnation" (hypercalcification), three cases of abnormally high blood-phosphorus, and several of hypercalcaemia, two of the latter showing clinical disturbances which were rectified when the irradiated ergosterol was omitted.

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