XXXV. HYPERVITAMINOSIS AND VITAMIN BALANCE.

PART II. THE SPECIFICITY OF VITAMIN D IN IRRADIATED ERGOSTEROL POISONING.

PART III. THE PATHOLOGY OF HYPERVITAMINOSIS D.

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PART II. THE SPECIFICITY OF VITAMIN D IN IRRADIATED ERGOSTEROL POISONING.

In the course of earlier papers Harris and Moore [1928, 1, 2] confirmed the toxic action of irradiated ergosterol in excess and concluded that "a weight of evidence pointed to the toxic action of excess of vitamin D *per se*." Recently it has been suggested, however, that the poisonous effects are not due to the vitamin, but are confined to certain preparations in which poisonous by-products have been allowed to develop. While we have not wished to deny the possibility of such additional toxic by-products we continue in our belief that vitamin D is the main and essential toxic ingredient present in irradiated ergosterol. Additional evidence in support of this conclusion is described in this communication.

Doubts have been raised on several occasions as to the reality of the parallelism between vitamin content and toxicity. Thus after we had completed in June, 1928, a series of observations upon solid irradiated ergosterol, prepared by activation in alcoholic solutions, it was suggested to us by Messrs British Drug Houses, Ltd., who had kindly supplied the specimen, that toxicity was peculiar to samples which had been prepared in this way. Experiments in their laboratories had in fact independently confirmed the toxic action of ergosterol when irradiated in alcohol, but toxic effects had never been observed in ergosterol activated by the routine method employed for "radiostol," a commercial preparation manufactured without the use of alcohol. In the same direction Dixon and Hoyle report [1928] that using ergosterol irradiated in oil by Messrs British Drug Houses, Ltd., they had been unable to confirm the alleged toxicity of irradiated ergosterol ("vigantol") as described by Kreitmair and Moll [1928]. In their conclusions they throw doubt on the assumption of hypervitaminosis, suggest instead that some independent toxic factor must have been present in the alleged toxic specimen of irradiated ergosterol, and aver that there is "evidence to show that when

irradiated in alcohol the product is toxic." Recently statements have appeared in the medical press that "radiostol" is irradiated in such a way as to give freedom from toxic by-products.

As a result of experiments with medicinal "radiostol," however, we have been able to demonstrate that such toxic properties are by no means restricted to samples of ergosterol activated by the alcohol method. Weight for weight, in fact, the irradiated ergosterol of "radiostol" has been found to be as poisonous in excess as the solid product used in our first experiment. We can only suppose that in previous experiments in feeding excess of these sources an insufficient allowance of the preparation was administered, or that administration was not continued over a sufficient period to allow the development of the more severe symptoms of hypervitaminosis.

EXPERIMENTAL.

1. *Non-toxicity of irradiated ergosterol in which vitamin D has been destroyed by prolonged over-irradiation.*

The result of this test was alluded to in our earlier paper, but experimental details have not yet been given.

The over-irradiated ergosterol was prepared from the same specimen of irradiated ergosterol that was fed to a control group of animals, and proved toxic to them. It was exposed in a thin film in a porcelain dish for 24 hours at a distance of 30 cm.¹

Three groups of rats were taken weighing about 90 g., and from one litter. The same basal diet was fed to all animals, but supplemented in the case of the first group with non-irradiated ergosterol, in that of the second group with the irradiated² ergosterol, and in the third with over-irradiated ergosterol, at the same concentration in each case, 0.1 % (*i.e.* 10 mg. of ergosterol in a 10 g. daily ration). The basal diet was the same as that previously described [Harris and Moore, 1928, 2, p. 1463], and all animals in each group received in addition 0.75 cc. of marmite and 2 drops of cod-liver oil *per diem*.

Those animals receiving irradiated ergosterol lost weight rapidly, and showed the symptoms previously described, including loss of appetite, marked cachexia, and a 20 % diminution in heart rate, and died after 20 to 34 days: *post mortem* examination showed the symptoms typical of hypervitaminosis D to be described later, notably enormous deposits of calcium salts in various sites in the body. Those having over-irradiated ergosterol, on the other hand, were after 35 days growing at the same rate as the normal controls receiving non-irradiated ergosterol; there were no signs of ill-health, heart rates were

¹ The specimens of irradiated, over-irradiated, and non-irradiated ergosterol were assayed for their vitamin D activities (by examination of rib junctions and by X-ray pictures of the long bones) and it was shown that the over-irradiated ergosterol had been reduced to at least 1/1000 of the original activity of the irradiated ergosterol.

² Irradiated by the method previously described [Harris and Moore, 1928, 2].

normal, and on being killed and autopsied no abnormalities, macroscopic or microscopic, were seen.

Fig. 1. Loss of toxicity on destruction of vitamin D by over-irradiation.

2. Comparison of toxicity of oil-irradiated ergosterol ("radiostol") with alcohol-irradiated ergosterol.

Rats of about 40 g. from one litter were divided into three groups. One group was given a diet containing 0.025 % of alcohol-irradiated ergosterol. The second group was given a similar diet containing approximately the same number of units of vitamin D, but in this case provided in the form of "radiostol" (oil-irradiated). The third group served as normal controls receiving basal diets of the same percentage composition but from which the ergosterol

only was omitted. (All three diets contained: purified caseinogen 20 %, rice starch 55 %, salt mixture 5 %, and fat 20 %, the last ingredient being in the case of the controls plain arachis oil, in the case of the alcohol-irradiated ergosterol group arachis oil containing 0.125 % of accurately assayed ergosterol, and in the case of the "radiostol" group "radiostol" itself. All diets were supplemented with marmite, 0.75 cc., and cod-liver oil, 2 drops per animal *per diem*.)

Fig. 2. Comparison of toxicity of oil- and alcohol-irradiated specimens of ergosterol.

———— Alcohol-irradiated ergosterol (0.025 %).
 = = = = "Radiostol" (oil-irradiated) at same vitamin D level.
 ——— Controls without excessive vitamin D.

Average daily food intakes: alcohol-irradiated, 5, 5, 5 g. (moist weight); oil-irradiated, 5, 5, 5 g. (moist weight); controls, 6–12 g., increasing during expt.

The results showed that there was a remarkable identity in the degree of toxicity of the irradiated ergosterol at a given level of intake, irrespective of the form in which it was administered, either as "radiostol" or "solid" (alcohol-irradiated) ergosterol. The close parallelism of weight curves is noteworthy (see Fig. 2); it was impossible to distinguish one group of animals from the other by appearance, and both showed similar but slight changes in heart rate, much less marked in this case, of course, than when ergosterol was given at four times the concentration, as for example in Exp. 1 above (Fig. 1). Death occurred after the same lapse of time with "radiostol" as with alcohol-irradiated ergosterol. *Post mortem* both groups were found to be very emaciated, there was diminution in size of spleen, atrophy of thymus, and great deposits of calcium in kidneys, aorta, heart musculature and other sites including calculi in bladder in several instances. No abnormalities could be detected in the control "arachis oil" group.

3. *Ergosterol irradiated in absence of solvent.*

Through the kindness of Prof. J. C. Drummond we were fortunate in being provided with a specimen of ergosterol of more than average purity. This we irradiated in a thin film in an atmosphere of nitrogen in a quartz flask for 240 mins. at a distance of 20 cm. from a mercury-vapour lamp. The ergosterol thus irradiated was then incorporated in the "synthetic" diet previously described (at 0.1 % level) and fed to a pair of rats weighing 40 g. In spite of the fact that our procedure in the absence of any solvent was probably inadequate to produce maximal antirachitic activity—insufficient material being available in this instance for an exact assay—the animals resuming growth at almost normal rate after 14 days; nevertheless, when they were killed after 28 days histological examination by Mr J. R. M. Innes revealed the typical extensive calcium deposits in kidney, aorta, etc. referred to above.

DISCUSSION.

The reality of hypervitaminosis D.

The above chemical evidence is uniformly in favour of our belief that vitamin D *per se* is toxic when administered in large excess. We have shown that toxicity is developed in ergosterol by irradiation, but that it is destroyed with the vitamin by prolonged over-irradiation. Toxicity is absent again from ergosterol resinsed in such a way as to cause no formation of vitamin D, although such treatment might be expected to produce impurities similar to the by-products of irradiation [Harris and Moore, 1928, 2]. It would be a remarkable coincidence to find that the hypothetical toxic by-product followed the presence of vitamin D so closely and so invariably as this.

By proving the toxicity of preparations obtained by irradiation in oil, the supposed confinement of toxicity to "alcohol-irradiated" samples is definitely ruled out. Toxicity has been found to run parallel with vitamin D content no matter what mode of preparation has been adopted. Moreover, this toxicity is made manifest in the same ill-effects and in the same *post mortem* findings which in themselves are very suggestive.

The possibility of additional toxic substances being present in certain samples of irradiated ergosterol, however, obviously cannot be excluded.

It has been stated by Underhill [1928] that by using ergosterol irradiated in a non-volatile solvent results were obtained which confirmed our own with alcohol-irradiated ergosterol. Nevertheless he considers the deleterious effects to be not entirely due to vitamin D itself, since ergosterol irradiated under different conditions "and subjected to further manipulation may have much greater deleterious results." It is impossible to deny the existence of such hypothetical toxic by-products but we consider that the above chemical evidence considered in conjunction with evidence of the marked disturbances in calcium and phosphorus metabolism which are the foremost characteristic of

ALCOHOL-IRRADIATED ERGOSTEROL

Fig. 3. Toxicity of irradiated ergosterol at different vitamin D levels. (See Part III.)

irradiated ergosterol poisoning (see Part III) amply justify our belief that the conditions described constitute a genuine state of hypervitaminosis D.

In Part III possible reasons are considered for the failure of several investigators to obtain symptoms of hypervitaminosis.

PART III. THE PATHOLOGY OF HYPERVITAMINOSIS D.

As to the pathology associated with excessive vitamin D intake, two opposing conclusions have so far been put forward.

On the one side, Pfannenstiel [1927] had found that rapid loss of weight and death were caused in his experimental animals, and Kreitmair and Moll [1928] in confirming this described the presence of massive deposits of calcium in many sites in the body (in the blood-vessels, heart musculature, kidneys, lungs and other organs).

On the other side, Dixon and Hoyle [1928], who failed to confirm the German work and were led to throw doubt on the assumption of hypervitaminosis, found no definite symptoms (except occasional haematuria) and when the animal was killed for *post mortem* examination they found in the organs neither pathological changes in the microscopical sections nor deposits of calcium, nor pathological lesions—except those associated with urinary calculi, which were found in every case (except one) at the highest level of vitamin D feeding¹.

Our own experimental animals, on all of the various high vitamin D diets already described, have at *post mortem* examination invariably shown the condition described by Kreitmair and Moll. Urinary calculi have frequently been evident as well. Several new aspects of hypervitaminosis have also come to light, and we have been surprised to note the remarkably rapid cures, with considerable disappearance of calcium deposits, which occurred when the excessive vitamin D was withdrawn from the diet.

EXPERIMENTAL.

(1) p_H of faeces.

On certain diets the absence of vitamin D is known to cause an increased alkalinity of the faeces, and when the vitamin is again given the faeces revert, temporarily at least, to a more acid reaction [Zucker and Matzner, 1923; Jephcott and Bacharach, 1926; Redman, Willimott and Wokes, 1927; Yoder, 1927; Grayzel and Miller, 1928]. Moreover artificial acidification of the contents of the gut [Irving and Ferguson, 1925], or the addition of acid to the food [Zucker *et al.*, 1922] can alleviate the effects of vitamin D deficiency (*i.e.* low blood-Ca or -P or both). Since, then, vitamin D deficiency tends to result in diminished acidity of faeces, it seemed of interest to determine whether under some circumstances vitamin D excess might be accompanied

¹ Kreitmair and Moll had also found that in hypervitaminosis the spleen shrank to a small fraction of its normal size: Dixon and Hoyle obtained, in one group of rats, 20 % diminution in weight only.

by an increased acidity, just as we have found in other ways too that the results of vitamin excess are in fact the opposite to those of vitamin deficiency, *e.g.* blood-phosphate [Harris and Stewart, 1929] or blood-calcium, or degree of calcification.

The p_H determinations were made in the usual manner [Jephcott and Bacharach, 1926] by Miss E. Alchorne and Mr A. L. Bacharach, to whom we are greatly indebted. When the basal diet used was in a sense an unpromising one for our purpose—*viz.* one on which the reverse change (from acid to alkaline on a vitamin D-deficient diet) is not readily, or at all, attained—no decisive increase in faecal acidity was obtained. It is true that we were

Fig. 4. p_H of faeces. Each curve is the average for three animals. *Single line*=excessive irradiated ergosterol (0.1 %). *Double line*=non-irradiated ergosterol. Normal Ca—P ratio in diet.

No clear separation into two distinct p_H zones could be effected. Animals showed great fluctuation in faecal p_H from day to day. The mean p_H increased during the course of the experiment. (Out of a total of 15 daily sets of readings, the lowest p_H recorded was on 14 occasions from an animal in the "excess vitamin" group, and the highest p_H recorded was on 11 occasions from an animal in the "non-irradiated" group.)

successful in showing that there was some decrease in the average daily faecal p_H of the animals receiving excessive irradiated ergosterol over the controls receiving excessive non-irradiated ergosterol, yet individual animals in the irradiated group not infrequently had a faecal p_H just as high or even higher than individual animals in the non-irradiated group.

Further work is in progress using a diet on which the change of reaction with absence of vitamin D occurs more readily (modified Pappenheimer, low P—high Ca) also with high P—low Ca diets.

(2) Influence upon the heart beat.

In the course of a separate electrocardiographic investigation, now in progress in collaboration with Dr A. N. Drury, we have found that the production of hypervitaminosis is invariably accompanied by a progressive diminution in rate of heart beat. A fall of 20 % or more was observed at the level of

0.1 % of irradiated ergosterol, but much less at 0.025 % ("radiostol" and alcohol-irradiated ergosterol).

(3) *Other symptoms.*

The remarkably rapid loss in weight (comparable with that in starvation), frequent diarrhoea, lowered food intake, and marked cachexia, greatly raised blood-phosphate [Harris and Stewart, 1929], and urine literally saturated with calcium salts, have been referred to elsewhere.

(4) *Post mortem findings.*

We are deeply indebted to Prof. J. B. Buxton and his staff for carrying out *post mortem* examinations.

(a) *Controls.* Animals which were killed after receiving the various control diets, containing (1) non-irradiated ergosterol, (2) non-irradiated ergosterol resinised by heating, (3) over-irradiated ergosterol or (4) oily solvents alone, were well nourished and showed no abnormalities, macroscopically, or in microscopic section, except for the occasional presence in the kidney of separate minute calcareous spots, such as are not infrequently observed in the normal rat.

(b) *Irradiated ergosterol* ("radiostol," etc.). Closely concordant findings were obtained in all instances, and as a typical example, those resulting in cases of death from excessive "radiostol" may be cited¹.

(1) *Macroscopic examination* showed that the animal was very emaciated, with an entire lack of subcutaneous fat.

Spleen. One-half normal size (with atrophy of Malpighian bodies), regenerating upon cure.

Thymus. Atrophied and less than one-quarter normal size (in some cases it had practically disappeared), regenerating with cure.

Bladder. Contained (e.g.) 5 calculi² as large as pins' heads and gritty material³.

Calcareous deposits. Tangible deposits, visible to the naked eye, in kidneys (which were beset with white areas), in heart-muscle (whitish bands), in pyloric end of stomach, aorta (walls thickened and sclerosed), colon, etc.

This abundant calcification has been previously observed by Kreitmair and Moll, but the extensive changes in the thymus and the condition of the urine do not appear to have been previously noticed.

(2) *Microscopic examination.* Specimens were cut in each experimental group from the heart, aorta, kidneys, stomach, duodenum, spleen, thymus, etc. Detailed histological findings are presented elsewhere, but the main conclusions regarding calcification are typified in the case of animals on 0.025 % "radiostol," alluded to above.

¹ Level of intake equivalent to 0.025 % of highly active solid irradiated ergosterol in the diet.

² Identified chemically.

³ In rabbits the urine in the bladder had become cloudy with excessive calcium salts, as shown chemically.

Aorta. Extensive calcification in the tunica media, causing the elastic lamina to become separate and intima to be raised from underlying structure.

Kidneys. Extensive areas of calcification in medulla, cortex and pelvis; and casts in the ducts of Bellini.

Heart musculature. The small arteries of the muscle definitely thickened and calcified, in some cases forming a complete ring. Some of the bigger branches of coronary arteries show the whole arterial coat to be converted into one calcified mass obscuring all structural detail.

Cartilaginous tissues. Calcification had occurred.

Duodenum. Calcification in reticular tissues.

Small intestine. Calcification in progress.

The calcification of cartilage appears to have escaped notice hitherto.

(5) *Effects of age, and degree of vitamin excess.*

Age. In the first place, as might be anticipated, we find that there is always a tendency for an older animal to survive longer on any given vitamin excess than for a younger one.

Table I.

	0.1 % irradiated ergosterol.									
Initial weight of animal, g.	40	49	50	51	53	64	86	100	106	
Days elapsing before death	6	10	7	24	10	25	20	34	32	

Similarly, disturbances in levels of blood-phosphorus and -calcium appear to be less easily produced in the older animal [Harris and Stewart, 1929; Hess, Weinstock and Rivkin, 1928].

Degree of excess. More important are the effects of differences in the degrees of the excess of vitamin D fed. Now we find that irradiated ergosterol gives rise to definite hypervitaminosis at a level of 0.025 % of the diet no less than at 0.1 %. But at the latter concentration there occurs a remarkably rapid loss of weight, at the rate of some 1 or 2 g. *per diem*, which begins immediately, on the first day of the experiment. On the other hand, at the slightly lower concentration of 0.025 % there is a cessation of growth but little or no loss of weight, and there is a considerable lengthening of the period which elapses before the animal finally succumbs to the hypervitaminosis, compared with that on the fourfold concentration (see Fig. 3, Part II). The *post mortem* findings differ somewhat in the two cases. The more prolonged hypervitaminosis (on the lower concentration of irradiated ergosterol) tends to result, it is found, in greater accumulation of calcareous deposits in the form of urinary calculi. When, however, death occurs more swiftly following rapid loss of weight (on the slightly higher concentration of irradiated ergosterol) calcium deposits may be seen as before as white bands or spotted areas in the heart muscle, kidney, colon, etc., but there appears to have been—not unnaturally—less opportunity for the growth of large calculi.

DISCUSSION.

Nature of hypervitaminosis.

It would appear that there are two threshold values for vitamin D, a minimum and a maximum. Below the minimum, there result deficiencies in blood-phosphorus, or -calcium (or both), and a consequent deficient calcification: the primary factor is a diminished retention of these two elements. Above the maximum threshold value on the other hand, there result excessively high blood-phosphorus or -calcium (or both) and a consequent excessive calcification. To some extent, as we have seen, the blood-calcium in hypervitaminosis is prevented from reaching too high a level by increased precipitation of calcium deposits and by excretion.

Several other aspects of hypervitaminosis are still quite obscure. As to the meaning of the atropic changes in the spleen and thymus there is as yet little evidence, but the reverse changes have been reported in rickets and we are examining the problem also in relation to the extirpation of these organs. The observed rapid fall in weight, with the suggestion of increased general metabolism, must also call for further enquiry, in the process of which we may reasonably expect some light to be thrown on the mode of action of vitamin D in the body.

Comparison of divergent results of different workers.

Our findings confirm those of Kreitmair and Moll [1928] that hypervitaminosis D is associated with gross disturbances in calcium metabolism. It remains to consider why other workers feeding large overdoses have failed to observe similar disturbances.

In the experiments of Dixon and Hoyle [1928] ergosterol which had been irradiated in oil was administered to rats at levels of 1, 2, 11 and 17 mg. *per diem* without giving rise to the symptoms of hypervitaminosis described by Kreitmair and Moll. When the 17 mg. level was reached, however, they did observe urinary calculi in their experimental animals. Their results also show that, while at levels of 1, 2 and 11 mg. there was good growth rate, at 17 mg. growth was poor, in fact had practically ceased. It is interesting to speculate whether the toxic threshold of vitamin D dosage may not have just been reached, and whether had they increased the vitamin D activity yet further, another four times at least, definite symptoms of hypervitaminosis might not have appeared. In a similar way Rosenheim and Webster [1927], who mentioned that they had found irradiated ergosterol to be harmless to a rat at 10,000 times the minimum effective dose, had no doubt not quite reached that concentration of vitamin D which is definitely toxic—100,000 times the minimum effective dose was needed in our experiments to cause a rapid loss of weight.

The absence of marked symptoms of hypervitaminosis in experiments on the human adult may probably be explained on similar grounds. By feeding

irradiated ergosterol at a dosage of 8 mg. for 21 days Havard and Hoyle [1928] observed neither injurious effects nor any change in blood-inorganic phosphorus or blood-serum. Although Kroetz [1927] reported that by feeding 18–30 mg. of the material great changes in mineral balance¹ were induced, he also found that such doses were apparently harmless. One is tempted to think that if it were possible for a still larger quantity to be taken, and over a sufficiently prolonged period, some rise in calcium or phosphorus must ensue. In the adult rabbit we find that the first rise in blood-phosphorus on the toxic dose of 10 mg. per day may not occur until after a fortnight [Harris and Stewart, 1929]. Calculating on the admittedly rough basis of relative body weights² from the lethal dose for the rat (1–10 mg.) and for the guinea-pig (about 40 mg.) the equivalent for a human of 10 stone would be some 10 g. *per diem*, and it may well be that the amounts consumed by these investigators were insufficient to cause any definite rise, particularly when one recalls that the adult is less susceptible to these changes than the young animal.

Since Part I was published, confirmation of the phenomenon of hypervitaminosis D has appeared in the independent work of Collazo, Rubino and Varela [1929], who name the condition “vitaminismus.” We also learn privately of confirmatory evidence obtained in other laboratories.

SUMMARY.

PART II.

1. Evidence is presented in support of the belief that excessive vitamin D *per se* induces a condition of specific hypervitaminosis, apart from possible separate (but undemonstrated) ill-effects due to poisonous by-products, if any.

2. “Radiostol” (activated in oil) and irradiated ergosterol (activated in alcohol) when fed at the same vitamin D levels produced hypervitaminosis of the same degree of severity. Ergosterol irradiated in absence of any solvent also produced specific ill-effects.

3. Fuller details are given of tests showing that the toxicity of irradiated ergosterol disappears concurrently with the destruction of its vitamin D by over-irradiation, and that non-irradiated ergosterol and ergosterol which has been resinised without the production of vitamin D are both non-toxic.

PART III.

4. *Post mortem* examination after death from hypervitaminosis D showed not only enormous deposits of calcium in the heart musculature, kidneys, and other organs as noted by Kreitmair and Moll, but often in addition the presence of urinary calculi, which were reported in the conflicting result of

¹ The renal excretion of P, Cl and water was found to be much increased, together with a lowering of blood-P and some acidosis. The effect is certainly surprising since it is the opposite of that produced by administration of vitamin D to the rachitic infant.

² The lethal dose varies appreciably from one species to another.

Dixon and Hoyle to occur in the absence of any such generalised hypervitaminosis.

5. Features of experimental hypervitaminosis in rats and rabbits, now observed for the first time, include—in addition to a remarkably high blood-phosphate—a tendency towards diminished p_H of faeces, diminished heart rate and at death atrophy of the thymus.

6. It would appear that there are low and high threshold values for vitamin D, below and above which there are caused deficient and excessive values respectively of blood-phosphorus and/or blood-calcium and resulting calcification. Atrophy of the thymus and spleen may be contrasted with the enlargement or hypertrophy often seen in clinical rickets.

The feeding of the experimental animals has been carefully attended to by Mr A. Ward.

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