The Effects on Dogs of Large Doses of Calciferol (Vitamin D).

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A number of investigations have appeared to show some relation between the rise of blood calcium produced by excessive doses of vitamin D and the action of the parathyroid glands. Jones (1926) had shown that the administration of cod liver oil to dogs in large doses, for some weeks before removal of the parathyroids, prevented the usual onset of tetany and increased the length of survival, whereas similar treatment with the oil was ineffective after the glands had been removed. The result was in line with a suggestion which had already been made with reference to irradiation [Block and Faber (1925)] that the effects of excessive doses of the vitamin D on blood calcium were not produced by a direct action, but by stimulation of excessive output of the parathyroid hormone. Greenwald and Gross (1929) explained Jones' results in this way, and showed that even 300–400 mgm. of irradiated ergosterol did not definitely relieve tetany or raise the blood calcium, unless it was supplemented by adding calcium to the diet. The experiments of Hess and his co-workers [Hess and Lewis (1928); Hess, Weinstock and Rivkin (1929, 1930); Hess, Benjamin and Gross (1931)] seemed to point, on the whole, in the same direction. Their results showed that larger doses of irradiated ergosterol are needed to produce a rise of blood-calcium on a calcium-free than on normal diets, and that still larger doses are necessary if the parathyroids have been removed in addition.

These and other similar investigations appeared to show that the effects of vitamin D, when given in doses large enough to cause a toxic hypercalcæmia, may be produced in several ways : (1) when there is plenty of calcium in the food, large doses of the vitamin promote excessive absorption of calcium from the alimentary canal, or alternatively, diminished re-excretion into the bowel [Taylor and Weld (1932)], just as therapeutic doses bring back a defective absorption to within the normal range. (2) When calcium is deficient in or absent from the food, larger doses of the vitamin can still cause a hypercalcæmia by withdrawal of calcium from the tissues. The work of Kreitmair and Hintzelmann (1928), Baumgartner, King and Page (1929), György (1930),

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Harris and Innes (1931), and Shelling (1932, 2) has shown that under such conditions calcium is withdrawn from the bones. (3) When the parathyroids are removed from an animal on a calcium-free diet, very large doses of the vitamin can still produce hypercalcæmia [Jones, Rapoport and Hodes (1930) ; Shelling (1932, 1)].

The resemblance between the effects of vitamin D in great excess, on a diet poor in calcium, and those produced by excess of the parathyroid hormone—hypercalcæmia with decalcification of the bones—naturally reinforced the suggestion that the effects of the vitamin under such conditions were produced indirectly, by stimulating output of the hormone. The hypercalcæmia still produced by the vitamin, in adequate doses, after removal of the parathyroids by the ordinary operation, would in that case have to be attributed to presence of accessory masses of parathyroid tissue, which had escaped removal. This is the view adopted by Taylor, Weld, Branion and Kay (1931), as the result of an extensive series of experiments. They drew attention to the close similarity between the symptoms produced in dogs by toxic doses of irradiated ergosterol and of the parathyroid hormone respectively, and between the appearance of the different organs—gastrointestinal mucous membrane, lungs, etc.—in animals dying from the two kinds of intoxication. They further drew attention to the parallelism between the effects of overdosage with vitamin D, and with parathormone respectively, in a range of different species. In dogs subjected to simple removal of the parathyroids with the thyroid gland, the resulting tetany was promptly relieved by administration of a large dose of irradiated ergosterol, and repeated administration led to the ordinary symptoms of overdosage. When, in addition, all tissue was removed which might harbour aberrant parathyroids, from the angle of the jaw to the sternum on both sides, the tetany was much more resistant to treatment with vitamin overdosage, and the latter had to be continued much longer before, eventually, the animals succumbed with the usual signs and symptoms produced by such excess. They regarded these results as confirming the view that excess of the vitamin acts through stimulation of the parathyroids, on the supposition that, after the most complete dissection of the neck, aberrant parathyroid tissue, embedded in the thymus or other mediastinal tissue, might still be left. The assumption seems to be involved, that a remnant of parathyroid tissue, inadequate to prevent a fatal tetany without further treatment, and incapable even of removing tetany under the stimulus of the vitamin until a very large excess has been administered, is nevertheless able, under continued stimulation, to produce death from parathyroid excess. The

conception seems to us to present difficulties. It should be noted, further, that Taylor and his co-workers did not begin to administer the vitamin until tetany had appeared, and then gave it by the mouth, in spite of difficulties due to trismus and vomiting. Under such conditions there might be doubt as to the effective absorption of the doses administered.

There has been some doubt as to whether the toxic effects of overdosage with the mixed irradiation product are due to excess of the vitamin itself, or to other derivatives formed from ergosterol at the same time. Hoyle (1930) attributed the effects to toxic impurities, and Holtz and Schreiber (1930), Windaus (1930), Windaus and Auhagen (1931) have shown that the mixed product can be so treated as to give a preparation devoid of the therapeutic action, but still toxic in large doses ; though the evidence is not clear as to whether the toxic constituent pre-existed as such in the antirachitic mixture, or was produced by further change of the vitamin. The question has been at least partly answered by the isolation of the pure crystalline vitamin D, named calciferol in England [Askew, Bourdillon *et al.* (1931)] and vitamin D_2 in Germany [Windaus *et al.* (1931)]. Both sets of observers found that the pure substance showed, in excessive doses, a toxicity for rats and mice proportionate to its antirachitic activity in small doses. It was to be expected that the same would hold good for the toxic effects in dogs. The availability of pure calciferol enabled this point to be settled. Since the pure substance could be prepared with relative ease in a state of high dispersion in a watery medium, suitable for intravenous injection, it also afforded a better opportunity of testing the question, whether the condition of the gastrointestinal mucous membrane, seen after fatal doses, is due to local action, or is entirely secondary to the hypercalcæmia.

The following short series of experiments on dogs has been made to obtain further evidence on these various points. The calciferol used was generously placed at our disposal by the British Drug Houses, Ltd.

Methods.

For determinations of calcium and phosphorus, blood was obtained either by allowing it to drop from a puncture in the lateral ear vein, or with a syringe from the external saphena vein, which was also used for injections. Calcium was determined by Clark and Collip's (1925) modification of the Kramer-Tisdall method, and phosphorus by that of Fiske and Subbarow (1925). For intra-

venous injection a 3 per cent. solution of calciferol in alcohol was added, drop by drop with vigorous stirring, in the proportion of 3 c.c. to 9 c.c. of sterile dog serum. An opalescent dispersion was thus produced, which was warmed to body temperature and slowly injected. Such injections were usually tolerated without any immediate symptoms, but one dog vomited shortly after an injection. If such a dispersion is kept for days in the cold, the calciferol ultimately separates in small crystals, which cause intravascular clotting if injected. For administration by the mouth a 5 per cent. solution in olive oil was used, the small measured volume being delivered from a syringe on to the back of the tongue and completely swallowed.

Results.

1. *Normal Dogs.*—The object of these experiments was to determine whether pure calciferol would produce the toxic effects characteristic of excessive dosage with vitamin D in dogs, to obtain an idea of the dose required to produce these effects, and to afford a general comparison between the effects of intravenous and oral administration. No attempt was made to determine a precise lethal dose by either method. This would have required a large number of experiments, and the total quantity needed to produce a lethal effect would probably have been different with administration in a single dose, from that required with divided dosage. No special measures were taken to control the diet, the dogs receiving the ordinary ration of meat and biscuits. The relation between dietary calcium and dosage had been sufficiently studied by earlier observers with impure irradiation products. For our simple objects a few experiments gave the information sought. To avoid large numbers we have expressed the doses in milligrammes of calciferol. These can be converted into international units of vitamin D activity on the basis of 40,000 units per milligramme.

Dog 1.   8·9 kgm.

February 1 ..   50 mgm. calciferol given intravenously.

The only symptoms observed were some lassitude and loss of appetite on February 3. Otherwise the dog remained lively and ate well. The stools, however, became loose and remained so, with occasional blood, for 2 weeks after the injection. The serum-calcium, initially 10 mgm. per cent., showed only the relatively small rise to 12·6 mgm. per cent. for a few days.

Dog 2.   8 kgm.

February 4   ..   100 mgm. calciferol intravenously. Serum calcium, 11·5 mgm. per cent.

February 9   ..   Dog, which had not been obviously abnormal, refused food and lost activity.   Weight 6·9 kgm.   Serum calcium, 18·3 mgm. per cent.

February 10 ..   Serium calcium, 19·5 mgm. per cent.

February 11 ..   Apparent improvement of condition and appetite. Serum calcium, 18·7 mgm. per cent.

February 12 ..   Dog had vomited in the night and showed renewed anorexia and lassitude. Serum calcium, 18·5 mgm. per cent.

February 13 ..   Dog becoming weak.   Weight 5·95 kgm.   Killed with chloroform.   Serum calcium, 17·5 mgm. per cent.

Post-mortem.—Nothing definitely abnormal in lungs or liver. Gastro-intestinal mucosa mildly congested. An ulcerative condition of the gums, which must have been present undetected at the beginning of the experiment, was evidently progressive. The rapid loss of weight and condition must, however, be associated with the high serum calcium, which rose to 19·5 mgm. per cent. on February 10, and fell slowly to 17·5 mgm.   The dose appeared to be near the lethal limit for a dog of this size.

Dog 3.   7·8 kgm.

February 8   ..   50 mgm. calciferol intravenously.   Serum calcium, 10·7 mgm. per cent.

February 9   ..   50 mgm. calciferol intravenously.   Serum calcium, 12·9 mgm. per cent.

February 10 ..   50 mgm. calciferol intravenously.   Serum calcium, 18·1 mgm. per cent.

Total calciferol, 150 mgm. over 2 days.

The serum calcium rose sharply and had reached 20·8 mgm. per cent. on February 11.   By this time the dog showed the usual symptoms of lassitude, weakness, anorexia and passage of loose fæces with blood.   At 5 p.m. on this day the dog, which was obviously going to die, was killed under an anæsthetic.

Post-mortem.—Gastro-intestinal mucosa and lungs distinctly, but not severely, congested. Serum calcium, 20·5 mgm. per cent.

These three experiments showed that the lethal dose of calciferol, with a single intravenous injection, or a few injections over 2 days, was in the neighbourhood of 100–150 mgm. for these dogs. The dose probably varies with the age of the dog, and it is unlikely that it can be expressed merely in terms of body weight. With this reservation, the lethal dose can be taken as about 12–20 mgm. per kilogramme. The following experiment shows the effect of a single much larger dose.

Dog 4.   8·7 kgm.

February 8	..	400 mgm. calciferol intravenously. Serum calcium, 10·5 mgm. per cent.
February 9	..	Apparently normal. Serum calcium, 15·5 mgm. per cent.
February 10	..	Less active, appetite poor. Weight 8·35 kgm. Serum calcium, 17·2 mgm. per cent.
February 11	..	Has vomited and passed blood *per anum* during the night. Very weak. Weight 7·65 kgm. Dies at 10·45 a.m.

Post-mortem (immediate).—Serum calcium, 19·5 mgm. per cent. Mucosa of stomach and intestine swollen and intensely congested with hæmorrhage into the lumen. Liver much congested; lungs less so. The blood was very viscous, and was found to contain 60 per cent. of corpuscles by volume; Hb = 157 per cent. by the human hæmoglobinometer (Haldane).

The next experiment shows the effect of repeated doses by the mouth.

Dog 5.   15 kgm.

February 8	..	50 mgm. calciferol by mouth. Serum calcium, 10·6 mgm. per cent.
February 9	..	50 mgm. + 60 mgm. by mouth. Serum calcium, 13·5 mgm. per cent.
February 10	..	80 mgm. by mouth. Serum calcium, 19·3 mgm. per cent.
February 11	..	Serum calcium, 18·2 mgm. per cent.

Total, 240 mgm. over 2 days.

This dog showed slighter symptoms of intoxication than those treated by intravenous injections, though it vomited on occasion. The serum calcium, however, had risen to 19·3 per cent. already on February 10, and the dog died early on February 12. The usual post-mortem appearances—congestion of the alimentary mucosa and of the lungs—were present in moderate degree.

The dose, it will be seen, amounted to 16 mgm. per kilogramme.

These experiments showed that pure calciferol, in excessive doses, produces the typical fatal hypercalcæmia in dogs produced by the mixed irradiation product. Intravenous injection appeared to be neither more nor less effective than oral administration in producing the fatal intoxication. The rapidity of onset of the symptoms and of the fatal termination was not noticeably different by the two methods. Dog 3 and dog 5, which probably received not much more than the fatal quantity, given over 2 days in each case, but intravenously in the one case and by mouth in the other, both died on the fourth day. It should be noted, in particular, that effects on the alimentary mucosa were as severe with intravenous as with oral administration ; the congestion was, therefore, secondary to a systemic condition, presumably associated with the hypercalcæmia, and not to a local action on the mucosa.

Effects after Parathyroid Extirpation.

Here again only a few experiments have been performed. Our object was to test the evidence which has been supposed to support the view that excessive doses of the vitamin produce their effects through the parathyroids. For this purpose it seemed to us undesirable to allow the effects of deprivation to appear, before the administration of calciferol was started. If calciferol acted by stimulating the parathyroids, it should fail to act when these glands had been removed, even though there had not been time for hypercalcæmia and tetany to appear. On the other hand, if the hypercalcæmia due to calciferol had begun to appear, its progress should be arrested by complete extirpation of the parathyroids.

Dog 8.   11·8 kgm.

February 22   ..   The dog was given 100 mgm. calciferol by mouth at 2.50 p.m. ; it was then at once anæsthetised with ether, and a simple extirpation of both lobes of the thyroid with attached parathyroids was performed aseptically, the operation being completed by 4 p.m. The animal had, therefore, lost its main parathyroid supply long before the initial dose of calciferol had been absorbed, and at least a day before any definite rise of the blood calcium would have been expected in a normal dog similarly dosed. Serum calcium at end of operation, 11·6 mgm. per cent.

February 23 .. 10 a.m.—25 mgm. calciferol by mouth.

5 p.m.—25 mgm. calciferol by mouth.

Serum calcium, 13·2 mgm. per cent. Condition apparently normal.

February 24 .. 10 a.m.—25 mgm. by mouth.

5 p.m.—25 mgm. by mouth.

Total, 200 mgm. or 17 mgm. per kilogramme. Serum calcium, 16·8 mgm. per cent. Dog seems apathetic.

February 25 .. 10 a.m.—Very weak and lethargic. Has vomited much during night. Serum calcium, 17·4 mgm. per cent.

12 noon.—Dies. Serum calcium, 16·2 mgm. per cent.

Post-mortem.—Lungs and alimentary mucosa mildly congested. Some free blood in intestinal lumen.

In the onset of symptoms, the rapidity of their fatal termination, and in the post-mortem appearances, this dog clearly showed no significant difference from those receiving similar doses of calciferol without removal of the parathyroids. The blood calcium also rose as promptly; the only apparent difference being that the level reached at the fatal termination (16–17 per cent.) was lower than in the unoperated dogs (19–20 per cent.). On the other hand, the result was not seriously different from those which Taylor and his colleagues obtained with this limited operation, and attributed to the presence of aberrant parathyroids. It was necessary for us to try the effect of a more complete exclusion. Two experiments were therefore made, in which the complete dissection of the neck, from the surface of the submaxillary gland above to the sternum below, was carried out, in addition to wide excision of the thyroid and attached parathyroids. Every suspicious fragment of tissue was removed, in accordance with the method of the Canadian authors, the lymphatic glands in relation to the submaxillary glands being excised as a further precaution.

Dog 9. 14·2 kgm.

In this experiment a large dose of calciferol was given on the day *preceding* the operation. We wished to observe whether the parathyroidectomy would interfere with, or delay, the hypercalcæmia.

February 25 .. Serum calcium, 12·3 mgm. per cent.

10 a.m.—100 mgm. calciferol by mouth.

5 p.m.—50 mgm. calciferol by mouth.

February 26 ..   10 a.m.—Serum calcium, 15·3 mgm. per cent.

11 a.m.—Anæsthesia with Dial intraperitoneally, supplemented by ether as required.

1 p.m.—Aseptic removal of all parathyroid tissue in the neck completed.

7 p.m.—40 mgm. calciferol by mouth.

Total, 190 mgm. (13·4 mgm. per kilogramme) over 33 hours.

500 c.c. warm milk with 10 gm. sugar by stomach tube at intervals after the operation.

February 27 ..   6.30 a.m.—Found dead.

Post-mortem.—Alimentary mucosa severely congested. Serum calcium, 15·3 mgm. per cent.

The operation does appear to have stopped the upward course of the calcium curve, but not to have delayed the fatal outcome, with typical effect on the mucosa.

Dog 10.   13·2 kgm.

In this case the first dose of calciferol was given, intravenously, immediately after the operation.

February 29 ..   10.30 a.m.—Serum calcium 12·2 mgm. per cent.

10.45 a.m.–12.15 p.m.—Complete excision from neck of thyroid and all tissue possibly harbouring parathyroids, under Dial and ether anæsthesia.

12.30 p.m.—100 mgm. calciferol intravenously.

6 p.m.—Serum calcium, 8·8 per cent.

3 raw eggs and 10 gm. sugar by stomach tube, with 300 c.c. of water.

March 1   ..   11 a.m.—Serum calcium 12·1 mgm. per cent.

50 mgm. calciferol intravenously.

Dog still drowsy from Dial.

5 p.m.—Takes 30 gm. fish and 50 gm. meat and water freely. Serum calcium, 12·8 mgm. per cent.

12 midnight.—Serum calcium, 15·5 mgm. per cent.

March 2 .. 11 a.m.—Serum calcium, 15·6 mgm. per cent. Weakness and anorexia.

50 mgm. calciferol by mouth.

Total 200 mgm. or 15·2 mgm. per kilogram.

4 p.m.—Serum calcium, 16·5 mgm. per cent.

11 p.m.—Serum calcium, 17·8 mgm. per cent.

March 3 .. Diarrhœa, with blood. Much weaker. Food given by tube immediately vomited.

10 a.m.—Serum calcium, 19·1 mgm. per cent.

4 p.m.—Serum calcium, 19·4 mgm. per cent.

11.30 p.m.—Serum calcium, 17·2 mgm. per cent.

Dies during night. Severe congestion and consolidation of the lungs, and pronounced hæmorrhagic congestion of whole gastrointestinal mucosa.

It will be seen that in this dog the blood calcium had fallen from the rather high normal of 12·2 mgm. per cent. to 8·8 mgm. per cent. in the 5½ hours which followed the completion of the parathyroidectomy, in spite of the fact that 100 mgm. of calciferol were injected at the end of the operation. The effect of parathyroid deprivation was, therefore, initially ahead of that of calciferol. By the next morning the blood calcium had returned to the normal, and thenceforward, with further doses of calciferol up to the normal fatal limit, rose continuously, death occurring on the fourth day with symptoms and post-mortem findings quite similar to those seen in the normal animal receiving a similar dosage.

The possibility was considered that the use of a stable anæsthetic, such as Dial, might complicate the result, by reducing the resistance of the tissues to the calciferol action, and thereby accelerating its effect. One further experiment was therefore made, in which all possible parathyroid tissue, accessible without opening the chest, was removed by an operation similar to the foregoing, but under pure ether anæsthesia.

Dog 12. 14 kgm.

July 25 .. 10.30 a.m.—Blood from saphena vein—serum calcium, 13 mgm. per cent.

10.45. a.m.—Operation started under full ether anæsthesia. Thyroid with attached parathyroids, lymphatic glands, and all tissue possibly harbouring accessory parathyroids

completely removed, from the submaxillary glands to the pleura.

11.45 a.m.—Operation completed and suturing begun.

12 noon.—150 mgm. of calciferol intravenously.

12.15 p.m.—Dog begins to recover from ether.

6.30 p.m.—Serum calcium, 9 mgm. per cent.

July 26   ..   Animal fairly normal and active.

10.30 a.m.—Drank 150 c.c. of milk in which 120 mgm. of calciferol were emulsified.

10.45 a.m.—Serum calcium, 11·5 mgm. per cent. Drank 200 c.c. milk with 20 gm. glucose.

12 noon–2 p.m.—Vomited hair and mucus, with a little milk clot.

2.30 p.m.—Ate a little meat, but vomited it shortly afterwards.

5.30 p.m.—40 mgm. of calciferol in oil placed on the back of the tongue and swallowed.

Total administered, 310 mgm.

July 27   ..   Dog fairly strong and active. Respiration somewhat laboured. Had drunk water during the night, and passed loose fæces, but had not eaten.

10.45 a.m.—Serum calcium, 16·2 mgm. per cent.

11 a.m.–1 p.m.—Vomited clear, bile stained fluid.

1–2 p.m.—Passed loose fæces.

5.30 p.m.—Serum calcium, 17·6 mgm. per cent. Refused offer of raw meat.

10.30 p.m.—Somewhat weaker. Serum calcium, 17·7 mgm. per cent.

July 28   ..   Dog still weaker. As it seemed likely to die before the next morning, it was killed under ether at 2.30 p.m.

Post-mortem.—Gastric mucosa slightly congested, that of the intestine strongly so. Lungs distinctly but not extremely congested. Serum calcium, 19·6 mgm. per cent.

It was clear then, that the absence of the stable anæsthetic did not weaken the toxic action. The serum calcium had risen to nearly a lethal value in 3 days, and the animal would have died within a further 24 hours, if the intoxication had been allowed to follow its course. The total quantity administered was 310 mgm. of calciferol, but it may be supposed that at least

30 mgm. of this were lost by vomiting, so that not more than 280 mgm., or 20 mgm. per kilogram, are likely to have been absorbed.

Several attempts were made to ensure an even more certainly complete removal of the parathyroids by proceeding, after the radical removal in the neck, to open the chest by splitting the sternum and remove the thymus and attached tissue completely. In two such cases it was necessary to insert a tracheal tube and perform artificial respiration, since the thymus could not be removed completely without opening the pleuræ ; and in these cases, although the chest was successfully closed, with re-expansion of the lungs and effective resumption of normal respiration, death occurred before recovery from the stable anæsthetic (Dial), and before the result could be attributed to the calciferol. In these cases the lungs were found to be massively congested. It seemed possible that their exposure in operation had rendered them abnormally liable to the action of the calciferol, but the experiments could not be regarded as having clear evidential value. In a third dog we were fortunate in finding an anatomical relationship which enabled the thymus to be completely removed after splitting the upper part of the sternum, without opening the pleuræ or interfering with the natural respiration.

Dog 11. 10 kgm.

April 27   ..   11 a.m.—4 c.c. Dial intraperitoneally.

2 p.m.—Serum calcium, 11·3 mgm. per cent. Operation under Dial anæsthesia, with ether as required.

3.10 p.m.—Completion of radical extirpation in neck. 1 c.c. Dial intraperitoneally.

3.15 p.m.—Sternum split and thymus completely removed without opening pleuræ.

4 p.m.—Removal of thymus complete. Suturing begun.

4.20 p.m.—150 mgm. calciferol intravenously.

5.30 p.m.—20 gm. glucose in 250 c.c. water, and 100 mgm. calciferol, given by stomach-tube.

Total calciferol, 250 mgm. or 25 mgm. per kilogram.

April 28   ..   10.30 a.m.—Dog conscious, but drowsy. Drinks water. Serum calcium, 12·9 mgm. per cent.

12 noon and 2.20 p.m.—Takes two raw eggs beaten up with 20 gm. glucose and water.

4.15 p.m.—Loose motion.

4.45 p.m.—Serum calcium, 15·2 mgm. per cent.

April 29   ..   7.30 a.m.—Becoming very weak. Has vomited bile-stained fluid.

              8.30 a.m.—Vomits.

              9.10 a.m.—Serum calcium, 15·0 mgm. per cent.

              1.30 p.m.—Dies.

Post-mortem.—Large clot in the right side of the heart. Lungs intensely congested, with fluid in the pleural cavities. Mucous membrane of the stomach shows the typical intense congestion. No remains of the thymus could be found.

This dog had received a total quantity of calciferol somewhat in excess of the lethal dose for a normal dog. There is no reason, however, to suppose that a smaller quantity would not have been effective, and the dog died, with the characteristic symptoms, in less than the usual period after the beginning of the administration—2 days instead of 4.

Discussion.

The only difficulty presented by these results is that of reconciling them with those recorded by Taylor, Weld, Branion and Kay (1931), and with the view which they based on them, attributing the toxic effects of vitamin D in excess to stimulation of parathyroid activity. Our dogs, after removal of the parathyroids as complete as that carried out by these authors, and in one case possibly even more complete, succumbed to calciferol intoxication not less readily than normal dogs. The difference in result is doubtless due to difference in procedure. Taylor and his colleagues waited for the full development of tetany before beginning the administration of irradiated ergosterol. This would have the possible advantage of providing direct evidence of the success of the removal. On the other hand, it would have the effect of producing a low starting level of blood calcium, with a corresponding delay in reaching a toxically high level after treatment was begun. We proceeded on the assumption that, if calciferol acted through the parathyroids, resistance to its toxic action ought to be produced immediately by removal of those glands, and without waiting for the symptoms of deprivation to appear. In two cases (dogs 10 and 12), it will be seen that a determination of serum calcium within 6 hours of the extirpation, and of the immediately following injection of calciferol, showed the fall characteristic of parathyroid loss, with calciferol already circulating. The effect of parathyroidectomy was quicker in onset than the opposite effect of calciferol, but the latter overtook it and prevailed. The only

difference which the absence of the parathyroids appeared to make to the toxic action of calciferol in our series, was to lower somewhat the level of blood calcium at which the action became fatal. The effect, when it was obvious, was equivalent to lowering the base-line from which the hypercalcæmia started, without altering the toxic action which accompanied it. We have not sufficient data to justify a theory of this effect ; but such a result would be expected if the action of calciferol were to cause the appearance of abnormal concentrations of calcium in the alimentary mucosa and the lungs as well as in the blood, and if the removal of the parathyroids prevented the full rise of blood calcium, without similarly protecting the lungs and the alimentary mucosa from the accumulation of a toxic excess. In any case, such effects as we have obtained would appear to be fully explained on the supposition that removal of the parathyroids and excess of calciferol affect the blood calcium in opposite directions, without having recourse to the assumption that calciferol acts by stimulating the parathyroids.

Our results, and their differences from those of Taylor and his co-workers, would be rather more easily reconciled with a hypothesis that calciferol acts, not by stimulating excessive secretion of the parathyroid hormone, but by rendering the organism abnormally responsive to the action of what is already in circulation. On this view, calciferol administered immediately after removal of the glands, as in our experiments, would indeed be expected to produce its toxic action more readily than in those of the Toronto workers, who waited for the appearance of severe symptoms before beginning the treatment. There are details in the evidence, however, which are difficult to reconcile with this supposition. It would be expected, for example, that calciferol, administered directly after extirpation of the parathyroids, would show its full effect at once and a declining effect as the hormone became deficient. We find, on the contrary, that the injection of calciferol has no immediate effect on the fall of blood calcium following the extirpation, and only begins to show its action, and then a practically normal one, some hours later, when the fall of blood calcium due to hormone deficiency would be already well in evidence. If the administration of the vitamin is delayed until the symptoms of hormone deficiency are well established, it might be expected quickly to show its sensitising effect to the traces of hormone still remaining, and then to fail of further action as the last traces become exhausted. Taylor and his colleagues found, on the contrary, a delayed effect of the vitamin under these conditions, but one which progressed with continued administration, to death with the characteristic symptoms of vitamin excess.

It appears to us, accordingly, that neither of these alternative views, of a direct connection between calciferol intoxication and the action of the para-thyroid hormone, can properly be reconciled with the facts. The similarity of the symptoms and of the post-mortem appearances, produced by a fatal excess of either, seems to be adequately explained by the fact that a sufficient excess of calcium in the blood and tissues, whatever the cause, even if it is produced by continued infusion of calcium salts, has this characteristic result, as demonstrated by Collip (1926). Such modifications in the toxic effects of calciferol as parathyroidectomy produces—any delay in their appearance, increase of necessary dosage, lowering of the level of blood calcium at which death occurs—seem to us to be adequately explained on the simpler supposition that the effects of calciferol and of parathyroidectomy on the concentration of calcium ions in the blood, are in opposite directions, but not connected by any causal relationship.

The fact that calciferol possesses the same toxic action, in excessive doses, as the crude product of irradiating ergosterol, may seem difficult to reconcile with the evidence that the mixed irradiation product can be deprived of its therapeutic action and yet retain the toxic action in large doses. The structure of calciferol is not sufficiently known to justify any definite theory ; but the suggestion of Windaus (1930), that the therapeutic action of small and the toxic action of large doses are due to different groups or linkages in the molecule, and that a substance is produced, by appropriate treatment of the irradiation product, having the structural feature which conditions the toxic but lacking that which conditions the therapeutic effect, appears to meet the facts already known.

We gladly acknowledge valuable help received from our colleague Mr. T. A. Webster, in carrying out these experiments and considering their results.

Summary.

The following are our main results and conclusions :—

(1) The pure, crystalline vitamin D, calciferol, has, in excessive doses, the characteristic toxic action on dogs of the crude product of irradiation of ergosterol.

(2) The toxic action is produced by intravenous injection as well as by oral administration. The congestion of the alimentary mucosa, produced by a fatal dose, is equally pronounced with either method of administration.

(3) Complete parathyroidectomy does not prevent, or significantly hinder,

the fatal intoxication produced by large doses of calciferol.   At most it lowers the level of concentration reached by the blood calcium before death.

(4) The results lend no support to the suggestion that vitamin D in excessive doses acts by promoting secretion of the parathyroid hormone, or by rendering the organism more responsive to its action.

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