CXXXIV. ON THE FUNDAMENTAL NATURE OF PARATHORMONE ACTION.

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IN a preliminary paper [McGowan, Cunningham and Auchinachie, 1931], certain tentative conclusions were drawn regarding the intrinsic and essential nature of vitamin D action. A series of complete calcium and phosphorus balance experiments¹ on rabbits has since been carried out with the result that these provisional conclusions have been substantiated in full. The detailed description of these observations will be published in a subsequent paper. The chief deduction drawn at the time was that vitamin D produced its effect by breaking up the lipoids of the tissues and setting free phosphoric acid which was neutralised *in situ* by the calcium of the blood, thus bringing about local deposition of tricalcium phosphate. It should be noted that such precipitation is extra-vascular. A precipitation of calcium salts in various parts of the body following the injection of parathormone has been observed by various investigators [*e.g.* Taylor *et al.* 1931], and it seemed of interest to try to locate the exact site of this deposition. It was thought that this might throw some light on the mode of action of parathormone as also on the vexed question as to whether vitamin D acts primarily and solely through the intermediation of the parathyroid gland.

Because of an acquired familiarity with the histology of their organs, fowls were employed in the investigation: while, on account of their insusceptibility to parathormone [Taylor *et al.* 1931], large doses were administered daily to laying hens, the blood-serum of which has already, as is well known, a high calcium content. It was hoped by these means to obtain a definite result even in such a refractory animal.

The parathormone employed was prepared by Eli Lilly and Co., Indianapolis, U.S.A., according to Collip's formula and contained 20 units per cc. It was injected into the pectoral muscles. The fowls were kept in individual cages. They were fed *ad lib.* on a mash consisting of bran 2 parts, sharps 4 parts, maize 2 parts, crushed oats 1 part and soya bean meal $1\frac{1}{2}$ parts, to $10\frac{1}{2}$ cwts. of which mixture had been added 40 lbs. of a mineral mixture. They received also, *ad lib.*, a grain mixture consisting of equal parts of maize, wheat and oats: oyster shell grit and water were freely available.

¹ The analyses were carried out by Messrs William Godden, William H. Williams and Miss B. W. Simpson.

The calcium content of the blood-serum and the phosphorus content of the whole blood were determined from time to time¹, while, at the death of the fowls, sections were prepared from practically every organ and tissue of the body and treated by von Kossa's silver nitrate method for the detection of tricalcium phosphate.

A brief résumé of the findings in the various experimental fowls will now be given. The hens employed were all 2 year old laying White Leghorns.

Hen 611, brought into the cage from free range on 18. iv. 32: breast-bone quite straight, regular, thin and rigid²: no eggs had been laid by 20. iv. 32, when the calcium of the blood-serum was found to be 28.1 mg./100 cc. and the phosphorus of the whole blood 6.04 mg./100 cc.: 50 units parathormone were injected on this date and again next day when the fowl was seen to be rather dull. On 22. iv. 32 it was found to be nearly dead but blood was obtained which contained calcium 20 mg./100 cc. and phosphorus 12.8 mg./100 cc. On *post mortem* examination, the most notable features observed were the presence of small white patches of various sizes in the substance of the breast-muscles (the other muscles were not examined), the smearing of the whole surface of the peritoneal cavity with egg-yolk from a burst ripe ovum, and ovary and oviduct in full laying condition.

The nature of the white spots in the muscles is difficult to establish. In the patches, the muscle fibres were markedly swollen and showed considerable disintegration and necrosis. This subject will be touched on again later. As regards the egg-yolk in the peritoneal cavity, the most feasible explanation of its occurrence is that the egg, having burst from its follicle and having missed the oviduct, in its unprotected state is easily ruptured by the movements of the gizzard and intestine *etc.* There is no evidence to suggest that the bursting is brought about primarily by the parathormone, though it seems to have something to do with the failure to get into the oviduct.

The femur and tibia were very sclerosed and contained a very small amount of a pale marrow (hen originally was slightly anaemic). It may be stated here that in none of the experimental hens employed was there any evidence of osteoporosis or any change, atrophy or hypertrophy, of the parathyroids subsequent to injection³. The most important finding, however, was discovered microscopically on examining the sections which had been treated with silver nitrate. Deposits of tricalcium phosphate were found intravascularly in the following situations: capillaries of the lungs, especially at the periphery of the lobules near the chest walls, large veins of the liver, *artérioles à housse* of the spleen, large veins and arteries of the spleen (also in the *vasa vasorum* of these arteries) and the capillaries of the glomeruli of the kidneys (see Plate IV).

The distribution of the condition just described was very irregular in individual organs; and while in this fowl all the organs just mentioned were affected, in others of the series certain of these organs escaped. In every case, however, the lungs were involved. Unfortunately, the intestines of this bird were not examined for calcium deposition. The aorta was quite healthy. All the tricalcium phosphate precipitation found was intravascular with the exception of a trace in the tubules of the kidney and a slight occurrence in phagocytes in the periportal spaces of the liver.

Hen 621, brought into the cage from free range on 18. iv. 32: breast-bone normal: laid an egg on 19. iv. 32; and a shell-less one on 20. iv. 32: on this date the serum-calcium was 22.3

¹ These estimations were carried out by Dr D. W. Auchinachie and Mr A. R. G. Emslie.

² The condition of this bone has been regularly employed for some time as an index of the state of the skeletal system generally and has been found to be of very great assistance in this regard.

³ One has observed fairly frequently cases of laying hens on free range with marked osteoporosis and great hypertrophy and greatly increased activity of the parathyroids together with the production of shell-less eggs. As such fowls were receiving large quantities of calcium in their food together with calcium grit, the condition would seem to be due to a lack of absorption of calcium brought about possibly by too low acidity of the intestinal tract.

mg./100 cc. and blood-phosphorus 3.19 mg./100 cc.: she laid a shell-less egg on 21. iii. 32: 50 units parathormone were given on 20th, 21st, 22nd and 23rd. She looked somewhat dull on the 23rd and was found newly dead, at 9 a.m. on the 24th: blood taken at once from the heart contained 14.6 mg./100 cc. calcium. The *post mortem* condition resembled that of hen 611, especially in regard to the white spots in the muscles, the smearing of the peritoneum with egg-yolk and the condition of the ovary and oviduct. There was no osteoporosis and no change in the parathyroids.

Hen 8, brought into the cage from free range on 22. iv. 32: breast-bone normal: serum-calcium 20 mg./100 cc. and blood-phosphorus 2.38 mg./100 cc. No eggs were laid during the experimental period. 50 units parathormone were given on 22. iv. 32 and again on 23. iv. 32; and 20 units on 24th, 25th and 26th, all at 9.30 a.m.: the hen was very dull on the 26th and was killed by bleeding at 2 p.m. The blood-serum contained 14 mg./100 cc. calcium and the whole blood 4.8 mg./100 cc. phosphorus. Again the findings *post mortem* were much as in the two previous cases but it was noticed that all the muscles of the body showed the white spots previously alluded to. There was no osteoporosis and no change in the parathyroids. Microscopically, the changes in the tissues were as in the previous two but less marked than in hen 611.

Hen 319, brought into the cage from free range on 25. iv. 32: breast-bone normal: blood-serum contained 27.3 mg./100 cc. calcium and whole blood 3.9 mg./100 cc. phosphorus: on 26. iv. 32 20 units parathormone; on 27. iv. 32 laid an egg and was given 20 units: on 28. iv. 32 laid an egg and was given 20 units; on 29. iv. 32 no egg; was given 20 units; on 30. iv. 32, 1, 2 and 3. v. 32 no egg, but 20 units each day; on 3. v. 32 serum-calcium 21.0 mg./100 cc. and blood-phosphorus 4.4 mg./100 cc.; on 4, 5 and 6. v. 32 no eggs laid, but 20 units each day at 9.30 a.m. killed by bleeding at 2.30 on 6. v. 32: serum-calcium 31.2 mg./100 cc. and blood-phosphorus 5.1 mg./100 cc. In this case there were no white spots in the muscles, no smearing of peritoneum with egg-yolk but the ovary was in full laying condition and there was an egg, complete with shell in the shell gland. Microscopically, tricalcium phosphate was found nowhere except in the capillaries of the lung. There was no osteoporosis and no change in the parathyroids.

Hen 566, brought into the cage from free range on 2. v. 32: breast-bone normal: an accident prevented the estimation of the serum-calcium but the blood-phosphorus was 6.2 mg./100 cc.: on 2. v. 32 50 units parathormone; on 3. v. 32 laid an egg and was given 50 units; on 4. v. 32 given 50 units; on 5. v. 32 laid an egg, given 50 units; on 6. v. 32 laid no egg, given 70 units at 9.30 a.m.: at 2 p.m. 6. v. 32 killed by bleeding: serum-calcium was 23.0 mg./100 cc. and blood-phosphorus 6.75 mg./100 cc. *Post mortem*, there were no white spots in the muscles, no egg-yolk on peritoneal surface and ovary and oviduct were in full laying condition; there was no osteoporosis and no change in the parathyroids. Microscopically, tricalcium phosphate was found only in the capillaries of the lungs.

It has been noticed that hens 611 and 621, which showed deposition changes so markedly, had greedily swallowed the calcium grit when put into the cages. It was thought that a resultant hypercalcaemia, produced in this way, might have had something to do with the results obtained and a test was carried out in this regard on hen 321.

Hen 321, brought into the cage from free range at 9.30 on 4. v. 32: breast-bone normal: fed at once by hand, a large quantity of oyster shell grit: blood-serum-calcium 29 mg./100 cc. and blood-phosphorus 5.8 mg./100 cc.: fed oyster shell grit again at 2 p.m.; and at 4.30 given 100 units parathormone into breast muscles: at 9.30 a.m. on 5. v. 32 the hen was very ill: it was killed by bleeding: serum-calcium was 18.8 mg./100 cc. and blood-phosphorus 17 mg./100 cc.: *post mortem* there were no white spots in the muscles but the peritoneal surface was smeared with egg-yolk, while the ovary and oviduct were in full laying condition. There was no osteoporosis and no change in parathyroids. Microscopically, deposition of calcium phosphate was found in the lungs and in a few tubules of the kidney. No definite answer to the question therefore was obtained but it has to be noted that the time between the first injection of the animal and its death was short. The experiment could not be repeated as the supply of parathormone was exhausted.

At this stage it was deduced from the observations just described that the parathormone was, in all probability, producing a condition of supersaturation of the blood with tricalcium phosphate and that this substance was separating out in the quieter parts of the circulation, as for instance in the large veins of the liver, while further it was being sifted out of the blood by the ordinary capillaries, wherever they formed a complex network, as for instance in the lungs, and

the glomeruli of the kidney, and also by the sinusoids of the liver and *artérioles à housse* of the spleen. A consideration of the illustrations seems to indicate that this conception was justified. The hypothesis was however put to the test by the intravenous injection into a fowl of a suspension of Chinese ink in saline solution, made by rubbing the solid ink up with the saline in a mortar and subsequent levigation to get rid of the larger particles.

Hen 544, was brought in from free range and injected intravenously by the wing vein with 35 cc. of such a suspension. During injection the comb became quite livid but later regained its normal red colour. The hen was killed 4 hours after injection, having in the meantime laid an egg: sections were prepared from practically every tissue of the body. Microscopically, carbon particles were found in the capillaries of the lung, the sinusoids of the liver and the *artérioles à housse* of the spleen¹ (see Plate IV). No carbon particles were seen in the large veins and arteries of the liver and spleen or in the glomeruli of the kidneys: on the other hand a considerable number were found in the phagocytic cells of the bone-marrow, the distribution being different in these respects from that of the tricalcium phosphate deposited in parathormone-treated hens.

The mucosa of various parts of the intestine of this hen was examined and carbon particles were found in fair quantity, not however in the capillaries but in the phagocytic cells of the tissue surrounding the capillaries in the villi. In this regard it was unfortunate that the mucosa of the intestines of such reacting hens as 611, 621 and 8 were not examined. The negative results obtained from examination of hens 319 and 566 cannot be regarded as conclusive because there was so little precipitation of tricalcium phosphate anywhere in these cases. The occurrence of the carbon particles in the phagocytes in this situation is very suggestive of a possible method whereby insoluble materials, such as iron refuse, tricalcium phosphate *etc.* may be removed from the body, the mobile phagocytes conveying them through between the epithelial cells on to the surface of the intestine.

The lung in this Chinese ink experiment provided another suggestive appearance, in that there were extravasations of blood into the air spaces (see Plate IV). The most feasible explanation of this was an embolic infarction by the carbon particles. The occurrence may serve to explain the extravasations of blood that have been found in the lungs and intestinal mucosa [Taylor *et al.* 1931] of highly susceptible animals, such as the dog, in parathormone and vitamin D poisoning².

The observations made in this investigation do not lend support to the idea that vitamin D produces its action primarily and essentially *via* the parathyroid glands. In considering this point, the relevant occurrences in vitamin D poisoning seem to be somewhat as follows. In the first place, a production of free phosphoric acid, as the primary effect of vitamin D action, appears to occur in the tissues which, in turn, attracts calcium from wherever it can, the intestine or bones, for neutralisation purposes. This need for neutralisation is urgent and calcium may be withdrawn from the blood more quickly than it can be replaced from the above sources. Hence a hypocalcaemia may develop which could serve to stimulate the parathyroid to activity, with the liberation of further quantities of calcium from the bones.

A pure parathyroid action could thus replace the original vitamin D action, if the process got out of hand. It should be noted, however, that, in this suggested mode of action, the parathyroid activity is secondary to the vitamin D activity (which can take place, as has been frequently shown, in

¹ The injection of Chinese ink intravenously provides an excellent method for demonstrating the *artérioles à housse* and for distinguishing them from the Malpighian arterioles.

² In the latter, although the main action is supposed to be in the tissues, accumulation of insoluble tricalcium phosphate in the blood-stream, as is to be suggested immediately in this paper, is not excluded. Large amounts of tricalcium phosphate are also frequently found in the intestinal mucosa in vitamin D poisoning.

animals deprived of their parathyroids). Further, parathyroid action differs intrinsically from vitamin D action in that it produces intravascular phenomena alone.

At present the most likely mode of action of parathormone seems to be a stimulation of the resorptive activities of the osteoclasts of the bones [*cf.* Hunter, 1930].

It seems possible that one may have been fortunate in these experiments in the selection of such an insusceptible animal as the fowl; for the smallness of the results produced has permitted of clearer observation and interpretation. A greater accumulation of tricalcium phosphate, in all probability, would have obscured the exact site of its deposition. The insusceptibility of the fowl is, in all probability, to be attributed to the fact that the laying hen tolerates with impunity serum-calcium concentrations as high as 25-30 mg./100 cc.

If hen 321, on account of the short duration of the experiment, be excluded from consideration, it will be seen that hens 611, 621 and 8, which showed tricalcium phosphate deposition and white patches in the muscle, also had their peritoneal surfaces smeared with egg-yolk from a burst ovum. This last phenomenon, on the other hand, was absent from hens 319 and 566 which showed tricalcium phosphate deposition in the lungs only and no white patches in the muscles. The following may be an explanation of these occurrences. It seems possible that the escape of the ripe ovum from the ovary may set in motion a train of events for the completion of the egg, one of which would be the provision in the blood of an extra supply of calcium for the formation of the egg-shell. Where this process is consummated by the ovum travelling down the oviduct, the extra calcium would be got rid of in this manner but the case would be quite other where the ovum was burst in the peritoneal cavity.

Taking into account the irregularity of occurrence and distribution of the calcium deposition as a whole in relation to similar occurrences in the muscles together with the actual histological appearances of the white spots, it seems not unlikely that these may be embolic infarcts due to occlusion of capillaries by deposited tricalcium phosphate. This question however is left open in the meantime.

The calcium and phosphorus contents of the blood at various stages are recorded as found but no interpretation of them is attempted at present. It will be observed, however, that, in general, the blood-serum-calcium subsequent to several injections is lower than before injection.

SUMMARY AND CONCLUSION.

The injection of large doses of parathormone intramuscularly into laying hens has produced a deposition of tricalcium phosphate inside the vessels as the main result. This, in all probability, is brought about by the parathormone liberating calcium hydrogen phosphate from the bones by a direct stimulation of the activity of the osteoclasts. This appears to be its only mode of

action. Vitamin D however seems to be able to function in two ways, primarily, by liberating phosphoric acid in the tissues which attracts calcium from the intestine or bones by its acidity, and secondarily, by invoking the aid of the parathyroids through an induced hypocalcaemia.

The parathormone used in these experiments was supplied by Dr Clowes, Director, Eli Lilly Research Laboratories, to whom I wish to express thanks.

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DESCRIPTION OF FIGURES IN PLATE IV

Tricalcium phosphate appears deep black.

- Fig. 1. Hen 8. Parathormone injection; lung; silver nitrate; low power; showing very large quantity of tricalcium phosphate in capillaries at periphery of lobules.
Fig. 2. Hen 611. Parathormone injection; liver; silver nitrate; medium power; showing large quantity of tricalcium phosphate in sinusoids and encrusting the walls of the veins.
Fig. 3. High power; vein in Fig. 2 showing the jagged incrustation of tricalcium phosphate. "a," blood clot.
Fig. 4. Hen 611. Parathormone injection; kidney; silver nitrate; high power; showing tricalcium phosphate in the capillaries of four glomeruli.
Fig. 5. Hen 611. Parathormone injection; spleen; silver nitrate; medium power; showing several Malpighian corpuscles with their *artérioles à housse* picked out by their tricalcium phosphate content; note absence of tricalcium phosphate from splenic pulp.
Fig. 6. High power of Malpighian corpuscle of Fig. 5; showing tricalcium phosphate in arterioles.
Fig. 7. Hen 621. Parathormone injection; spleen; silver nitrate; high power; showing artery with walls encrusted with tricalcium phosphate (section somewhat oblique).
Fig. 8. Hen 611. Showing vein (seen in Fig. 5) with walls encrusted with tricalcium phosphate; note jagged nature at places.
Fig. 9. Hen 544. Chinese ink injection; lung; unstained; low power; showing carbon particles in capillaries at periphery of lobules; also extravasation of blood into air space (a).
Fig. 10. Hen 544. Chinese ink injection; liver; unstained; low power; showing sinusoids filled with carbon particles.
Fig. 11. Hen 544. Chinese ink injection; spleen; unstained; low power showing several Malpighian corpuscles with their *artérioles à housse* picked out by their carbon content: also comparative absence of carbon from the splenic pulp.
(Figs. 9, 10 and 11 are much less highly magnified than the corresponding figures for the parathormone experiments, viz. Figs. 1, 2 and 5.)