STUDIES ON THE EFFECTS OF CALCIFEROL IN THE THYROPARATHYROIDECTOMIZED-NEPHRECTOMIZED RAT

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Various views are held regarding the manner in which massive doses of irradiated ergosterol, or other antirachitic preparations, produce an elevation in the blood calcium. The most plausible among these views appear to be the following: (1) The antirachitic agent stimulates the parathyroids to great activity. (2) The antirachitic vitamin influences the net absorption of calcium from the intestinal tract and, therefore, affects the serum calcium level. (3) The antirachitic agent acts directly on the organic matrix of the bone, releasing calcium to the blood. (4) A toxic by-product of the irradiation of the sterol is responsible for the hypercalcemia which often results after the injection of massive doses of the antirachitic preparation.

In 1928 Hess and Lewis (2) offered evidence in support of the view, first advanced by Block and Faber (3), that irradiated ergosterol acts through stimulation of the parathyroid glands. Hess and Lewis stated that the hypocalcemia induced in dogs and monkeys by feeding diets low in calcium could be converted into hypercalcemia by means of irradiated ergosterol only when the parathyroids are intact, and not in their absence. This view was later abandoned when it was found by Hess, Weinstock, and Riv-

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1 Harris and Innes (1) define the term as follows: "Net absorption = Ca (or P) intake minus faecal output = absorption from gut minus excretion into gut."
kin (4) that hypercalcemia could be produced in the parathyroidectomized animal if the dose of irradiated ergosterol was sufficiently great. Jones (5), Wade (6), Shelling (7), Comel (8), Urechia and Popoviciu (9), Demole and Christ (10), Brougher (11), and Reed and Seed (12) have presented evidence which may be interpreted as supporting the latter view.

Taylor and his associates (13, 14) are of the opinion that due attention has not been accorded the possible presence of parathyroid “rests,” which may have been stimulated by the large doses of antirachitic agent used by many of the above workers. In support of their view they submitted their observations on two groups of dogs, one of which had been subjected to thyroparathyroidectomy, and the other to thyroparathyroidectomy and careful resection of all aberrant neck tissue. In the first group the vitamin D preparation alleviated the symptoms of parathyroid tetany within 1 hour after administration, but in the second group it was without effect. Dale, Marble, and Marks (15) have failed to obtain full confirmation of the experiments of the above workers. They found that their dogs developed a high level of calcium in the blood on the administration of the antirachitic agent as readily after removal of the parathyroids as before the operation. Workers in our laboratories (16, 17) have submitted evidence which minimizes the importance of accessory parathyroid tissue in the rat. They are of the opinion that various controllable factors, such as temperature, immediate dietary treatment, etc., are far more important than accessory parathyroids in preventing tetany in the rat.

Harris and Innes (1) contend that irradiated ergosterol tends to increase the absorption of calcium and phosphorus, or to diminish the “back absorption” of these substances into the gut. They believe that their experimental results are not inconsistent with the simple view that vitamin D acts by increasing the apparent solubility of Ca and phosphate in the blood.

Shelling (7) is of the opinion that the antirachitic agent acts directly on the organic matrix of the bone, releasing calcium to the blood. He has shown that hypercalcemia and calcification in certain vital organs can be produced by the oral administration of viosterol in parathyroidectomized rats which are on a calcium-free diet.
Tweedy and McNamara (18) have shown in the nephrectomized rat in which the parathyroids are intact that the serum calcium drops very gradually during the remaining life span (48 to 72 hours) of the animal, even though the serum inorganic phosphorus may reach 20 mg. per cent during the same interval (19). McJunkin, Tweedy, and McNamara (20) observed that in the above animal massive doses of calciferol produce hypercalcemia, if the injections are begun within 5 hours after nephrectomy when the serum calcium-phosphorus ratio is not greatly changed. They also observed slight to marked osteoclastic resorption in the femurs. If the injections were delayed longer than 5 hours after nephrectomy, bone resorption was sometimes observed, but hypercalcemia, if it occurred, was not present at the end of 48 hours. After the delayed injections, however, the serum calcium did not fall but increased slightly.

In the present work it will be shown that if the thyroparathyroidectomized rat is allowed to develop a low serum calcium-phosphorus ratio and is then nephrectomized, the subsequent injection of calciferol produces no apparent effects. Hypercalcemia is not produced, nor are there areas of resorption in the long bones. Thus removal of the kidneys appears to render the thyroparathyroidectomized rat immune to the action of calciferol, or to any toxic impurity that may be present in the antirachitic preparation. If, however, the serum calcium-phosphorus ratio of the thyroparathyroidectomized rat is converted into a normal value by the administration of a high calcium-low phosphorus diet, then nephrectomy does not prevent the production of hypercalcemia by calciferol.

Materials and Methods

Albino rats of the Wistar strain were used. These animals ranged from 60 to 120 days in age, and from the time of weaning were maintained on a diet, which consisted of Fox Chow (a commercial diet), supplemented by meat scraps, and cabbage leaf twice weekly. The Fox Chow contained about 1.36 per cent of calcium, and 0.98 per cent of phosphorus. The rachitogenic diet used was the Jones modification (21) of the Steenbock-Black rachitogenic Ration 2965 (22). This diet consisted of commercial
Calciferol

yellow corn-meal 66 parts, wheat gluten 20 parts, wheat embryo 10 parts, calcium carbonate 3 parts, and sodium chloride 1 part. Food was withdrawn after the animals were bilaterally nephrectomized, but they were allowed water during the postoperative period. In each case the animal was bedded on cotton, and kept at a temperature which did not vary greatly from 24°. Blood samples were taken by cardiac puncture, when the animals usually showed no more than a moderate degree of toxicity as judged by their activity. Serum calcium determinations were made by the Kramer-Tisdall method as modified by Tweedy and Koch (23), and serum inorganic phosphorus by Bodansky's method (24).

Recognition of bone resorption was not attempted except in connection with osteoclastic activity. A description of the histological technique employed is given in a previous paper (20).

Crystalline calciferol (40,000 I.U. per mg.) was dissolved in fresh Mazola oil just before being used, and injections were made, subcutaneously, with a 1 cc. tuberculin syringe.

EXPERIMENTAL

Fifteen rats (Nos. 1 to 15, Table I), which were thyroparathyroidectomized, had an average serum calcium of 6.49 mg. per cent 3 to 5 days after the operation, and in several other animals the serum inorganic phosphorus ranged from 8 to 10 mg. per cent. After being on the stock diet for about 1 month, these animals were removed from the diet and immediately bilaterally nephrectomized. The first injection of calciferol was made within 1 hour after nephrectomy, and the next two at 6 and 18 hours, respectively. In Table I are shown the individual serum calcium values 48 hours after nephrectomy. It will be seen that the serum calcium level in thirteen of the fifteen animals appears to have been unaffected by the calciferol.

Seventeen rats which had been placed on a low calcium diet immediately after thyroparathyroidectomy exhibited definite signs of tetany within 48 hours. These animals were placed on the Jones-Steenbock rachitogenic diet and remained on it for approximately 1 month. After this period of dietary treatment the serum calcium in nine of the animals (Group II, Table I) varied from 7.6 to 10.2 mg. per cent, with an average value of 9 mg. per cent, and the serum inorganic phosphorus in four of
these animals ranged from 4 to 5 mg. per cent. Five of these animals, and the remaining eight animals from which blood samples had not been taken, were continued on the rachitogenic

diet for 10 days. These thirteen animals were then removed from the diet and immediately nephrectomized. Eight were injected with calciferol, and five were not injected. 48 hours after nephrectomy blood samples were collected and analyzed

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<th>Rat No.</th>
<th>Serum Ca Before nephrectomy</th>
<th>Serum Ca After nephrectomy + vitamin D&lt;sub&gt;3&lt;/sub&gt;</th>
<th>Litter</th>
<th>Serum Ca, controls Before nephrectomy</th>
<th>Serum Ca, controls After nephrectomy</th>
<th>Bilaterally nephrectomized + vitamin D&lt;sub&gt;3&lt;/sub&gt; mg. per cent</th>
<th>Serum inorganic P mg. per cent</th>
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* 3 to 6 × 460,000 i.u.
† 3 × 460,000 i.u.
for calcium and phosphorus. In the animals injected with calciferol, the serum calcium varied from 10 to 19 mg. per cent, with an average value of 13 mg. per cent, and the serum inorganic phosphorus averaged 9.93 mg. per cent. The difference of 4 mg. per cent in the serum calcium of controls and injected animals shows that the calciferol which had been injected was effective in causing a mobilization of calcium into the blood.

In view of our observation that osteoclast activity was absent from the femurs of the injected animals, it appeared that the calcium mobilized by the calciferol might be drawn from the soft tissues, the food in the gastrointestinal tract, or from both the food and the soft tissues. The following experiment was then carried out. Eight rats were thyroparathyroidectomized and kept on the Jones-Steenbock rachitogenic diet for 30 days. 24 hours before they were nephrectomized, four of these animals were placed on a diet that was low in all mineral constituents in order to sweep excess calcium from the gastrointestinal tract. The other four animals were continued on the high calcium-low phosphorus diet until they were nephrectomized. Then three of the latter group were injected with calciferol in the same amount and manner used for the rats in Group II, Table I. At the end of 48 hours these three animals showed serum calcium values of 12.8, 13.4, and 14.7 mg. per cent, respectively. The un.injected animal showed a serum calcium value of 8.28 mg. per cent. In the four animals which had been changed to the low mineral diet, nephrectomized, and injected with calciferol as described above, we found the serum calcium to be 8.0, 8.1, 8.2, and 13.8 mg. per cent, respectively. These data clearly indicate that where calcium was present in the gastrointestinal tract of the animal it was absorbed, and accumulated in the blood if calciferol was injected.

**DISCUSSION**

It is a well established fact that rats which are maintained on a diet that is optimal for bone growth usually show a drop in serum calcium and a rise in serum inorganic phosphorus within 24 to 48 hours after parathyroidectomy. In the present experiments we found an average serum calcium of 6.49 mg. per cent, 3 to 5 days after thyroparathyroidectomy (Rats 1 to 15, Table I),
and in previous work (25) we have shown that this low level in the serum calcium may be present for 6 or 7 months before a return to low normal values is indicated. In several other animals, similarly treated, we found that the serum inorganic phosphorus is regularly 8 to 10 mg. per cent when the above level in serum calcium is reached.

Shelling (26) has shown that massive doses of viosterol greatly increase the excretion of calcium and phosphorus in normal rats, and that viosterol directs the excretion of the extra calcium and phosphorus away from the bowel towards the kidneys. In Rats 1 to 15 (Table I), the serum calcium was low, and the serum inorganic phosphorus was presumably high at the time the injections of calciferol were begun. Consequently it would appear that the amount of calcium entering the blood was greatly restricted by the more rapid accumulation of inorganic phosphorus in the blood. The rise in serum calcium above the control values in Rats 5 and 9 (Table I) may have been due to the calciferol injected, or it may have occurred during the interval between thyroparathyroidecetomy and nephrectomy. However, the absence of any evidence of osteoclast activity in the bones and the low serum calcium in thirteen of the fifteen animals clearly indicate that calciferol had little effect in mobilizing calcium into the blood.

In the animals of Group II (Rats 16 to 32, Table I), in which there was presumably a normal serum calcium-phosphorus ratio at the time of nephrectomy, the injection of calciferol caused an increase in the serum calcium of all the animals that were injected, and hypercalcemia in two of them. The fact that the bones of these animals showed the absence of more than the normal number of osteoclasts indicates that the primary action of calciferol was elsewhere. Furthermore, the failure of calciferol to produce an increase in the serum calcium of three of the four thyroparathyroidectomized rats from which the high calcium-low phosphorus diet was withdrawn 24 hours before they were nephrectomized, and the positive action of calciferol in the three animals left on the diet until nephrectomy, strongly suggest that the source of the extra calcium was the gastrointestinal tract.

Watchorn (27), Brown and Shohl (28), and Shelling (26) have
shown that irradiated ergosterol decreases calcium excretion in the feces and increases urinary excretion of calcium. Presumably in our thyroparathyroidectomized rats (Group II, Table I) that were nephrectomized and injected with calciferol, the accumulation of calcium in the blood was due to the action of calciferol in greatly restricting the fecal excretion of calcium. Since at the same time the fecal excretion of phosphorus was probably greatly decreased, its accumulation in the blood served to decrease, or to limit the absorption of calcium. The failure of the bones of these animals to undergo resorption may have been due to the retention of calcium to a greater extent than that which occurred in our nephrectomized rats in which the parathyroids were intact (20), and in which we found both bone resorption and hypercalcemia after the injection of calciferol. The present experiments strongly suggest that massive doses of calciferol act by decreasing the fecal excretion of calcium.

It would seem that our experiments support the view that the toxic action of calciferol arises through its effects on the calcium and phosphorus metabolism. Furthermore, it is suggested that the necrotic lesions of parenchymal tissues that often follow the injection of excessive amounts of calciferol are secondary to disturbances in the calcium and phosphorus metabolism.

SUMMARY

Administration of massive doses of calciferol to thyroparathyroidectomized-nephrectomized rats, in which there is a low serum calcium-inorganic phosphorus ratio, is ineffective in producing hypercalcemia. If, however, appropriate dietary treatment is instituted soon after thyroparathyroidectomy so that the normal relationship of calcium to phosphorus in the blood is restored, or maintained, then calciferol is effective in promoting mobilization of calcium into the blood of such animals after they have been nephrectomized.

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