

a determining factor in the toxicity and hypercalcifying property of viosterol. In the presence of a calcifying agent, increasing the amount of phosphorus in the diet renders the organism more susceptible to hypercalcification. With large doses of viosterol animals on certain diets may die without showing any signs of hypercalcification, although hypercalcemia may be present; while on other diets they may show hypercalcification without hypercalcemia.

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**Effect of Calcium and Phosphorus of Diet on Tetany and Serum Calcium of Parathyroidectomized Rats.**

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In the study of experimental hypoparathyroidism 2 factors frequently cause contradiction and confusion: (1) the anatomical relation of the parathyroid in the animals employed; and (2) the composition of the diet, especially with respect to its calcium and phosphorus content. In experiments with the dog or the monkey the extirpation of the parathyroids without destruction of thyroid tissue is frequently impossible and the complete operation of thyro-parathyroidectomy must be used. That the thyroid alone has a profound influence on calcium and phosphorus metabolism has long been appreciated and recently Aub<sup>1</sup> and his associates have demonstrated this fact by metabolism experiments in human beings. Hence the removal of the thyroid along with the parathyroids may cause an alteration in calcium and phosphorus metabolism different from that observed when either of them alone is removed. The rat, on the other hand, has but 2 encapsulated and easily removable parathyroids. Erdheim<sup>2</sup> and his followers made use of this anatomical fact and produced tetany in rats by cauterizing the parathyroids, and causing little or no destruction of the adjacent thyroid tissue. Erdheim showed that parathyroidectomy in the rat produces faulty dentition, delays calcification of a callus and impoverishes the body of lime salts.

The experiments of Erdheim were conducted prior to our more

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<sup>1</sup> Aub, J. C., Bauer, W., Heath, C., and Ropes, M., *J. Clin. Invest.*, 1929, **7**, 97.

<sup>2</sup> Erdheim, J., *Frankfurt f. Path.*, 1911, **7**, 175.

exact knowledge of complete diets and hence no control of calcium and phosphorus intake was considered. Even in recent times the diets used in experiments on dogs and monkeys have consisted mostly of meat and biscuit and hence have been of a high phosphorus and low calcium variety. The addition of milk to such a diet or the accidental finding of calcium in infusorial earth used in metabolism experiments is looked upon by many as a faulty procedure; and milk is frequently referred to as having detoxifying properties in relieving parathyroid tetany. That the beneficial effects are due to the high calcium content of the milk must be apparent, and its mode of action has thus been explained by Salvesen.<sup>3</sup> It is obvious that in the normal animal the parathyroids regulate the serum calcium level, even in the face of a very small intake, but, when this regulatory mechanism is removed the small amounts of the calcium ingested are insufficient to maintain a normal serum calcium concentration, especially when the phosphorus concentration becomes elevated and part of it must be either excreted or deposited as calcium salts.

With these facts in mind, the effect of the calcium and phosphorus or the diet on the tetany and serum calcium was studied in parathyroidectomized rats. The rats were of our own breeding stock, approximately middle-aged or young adults and in perfect health. Two rats were used for each group and the experiments repeated several times at different periods, so that more than 150 rats were used. The operation consisted of the surgical removal of the encapsulated parathyroids on both sides of the thyroid while the animals were under amytal anesthesia. The diets used were "complete" except for variations in calcium and phosphorus.

The results of these experiments may be summarized as follows:

(1) Animals kept on a low calcium, optimal\* phosphorus diet before operation developed severe tetany within 12 to 24 hours after operation. The tetany was very violent and many died within 24 to 48 hours in *status tetanicus*. Those kept on our stock diet until the operation and then placed on the low calcium diet weathered the operation much better and tetany came on more gradually, usually appearing within 24 to 48 hours. The tetany thus produced was chronic and the animals survived for months even on the low calcium diet.

(2) If, after the establishment of tetany, 1%  $\text{CaCO}_3$  was added to the low calcium diet, many of the animals gradually lost

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<sup>3</sup> Salvesen, H. A., *J. Biol. Chem.*, 1923, **56**, 443.

\* See footnote † in Paper No. 5279.

the symptoms of tetany; but severe tetany reappeared in about 5 days if the calcium was removed from the diet. The serum calcium levels in these groups varied from just above the tetanic level to as high as 12.8 mg. %.

(3) On the stock diet, tetany gradually disappeared and the serum calcium approached normal levels.

(4) On the Steenbock rachitogenic high calcium, low phosphorus diet the animals became free of tetanic symptoms very quickly and the serum calcium level varied between 11.9 and 12.9 mg. %.

(5) Animals kept on the low calcium and high phosphorus diet continued to have tetany for months and the serum calcium concentration remained at a tetanic level.

(6) On a high phosphorus and minimal\* calcium diet, another factor comes into play, that of a calcium retention in the body depots. Although the serum calcium concentration may be low, the tetany frequently disappears. This will be discussed in a subsequent paper.

(7) In a few large animals cardiac puncture was performed under amytal anesthesia and blood was obtained at intervals concomitant with the change in the calcium and phosphorus of the diet. The findings corroborated the clinical symptoms and the findings observed when other animals were sacrificed for such determinations in groups. Tetany and low serum calcium concentrations were observed when the animals were on a low calcium intake; and the tetany was alleviated, and the serum calcium level rose with increase in the calcium intake.

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### Effect of Viosterol on Serum Calcium of Parathyroidectomized Rats.

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Recently Hess<sup>1</sup> suggested that the effect of viosterol in raising the serum calcium level was through the parathyroids. This suggestion was based on his observations that in monkeys and dogs fed *large* doses of viosterol the serum calcium level frequently rose to 13-16

<sup>1</sup> Hess, A. F., Weinstock, M., and Rivkin, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **26**, 555.