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[1] 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

[1] Hess, A. F., Weinstock, M., and Rivkin, H., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 555.

mg. %. After thyroparathyroidectomy, however, *large* doses of viosterol failed to raise the calcium above the tetanic level. Greenwald[2] reports similar observations with cod liver oil and irradiated ergosterol. On the other hand, Jones,[3] Brougher,[4] Urechia and Popovicius[5] report that antirachitic agents, when given in large doses, are able to raise the serum calcium level and ameliorate the tetanic symptoms in thyroparathyroidectomized animals. The diets in these experiments are either not mentioned or are of an inconstant composition. Greenwald's diets were usually of the high phosphorus and low calcium type, and he attributes the beneficial results of vitamin D in one of his animals to an accidental mixture of calcium in the infusorial earth and that in Brougher's experiments to the administration of milk.

In the preceding paper[6] the importance of the calcium and phosphorus of the diet in relation to the serum calcium level after parathyroidectomy was stressed. In the present communication the effect of viosterol and of diet on parathyroid tetany is reported.

The results may be summarized as follows: 1. Low calcium and optimal* phosphorus diet. (a) When viosterol is added to this diet in dosages of 5% equivalent to cod liver oil† moderate elevation of the serum calcium may be observed after 20 days, and the animals continued on this diet with mild or no tetany for several months. The serum calcium levels ranged between 6.9 to 8.1 mg. %.

(b) When the dose was increased to 500%, the serum calcium levels were 7.0 and 8.3 mg. % in 24 days; 8.8 in 30 days and 10.6, 12.2 and 13.0 in 65, 73 and 90 days.

(c) With 20,000% viosterol the serum calcium was 11.0 mg. % in 8 days and the tetanic symptoms disappeared.

2. Minimal* calcium and phosphorus diet. (a) With 5% of viosterol the serum calcium level attained 11.0 mg. %. (b) With 500% viosterol, the serum calcium concentration was 7.6 and 8.4 mg. % and, within 3 months, it ranged between 8.6 and 12.6 mg. %.

A large animal of this group was bled by cardiac puncture on the second day after the operation and maintained on a low calcium diet. The serum calcium was 9.3 mg. % and tetany had not yet ap-

[2] Greenwald, I., and Gross, J., *J. Biol. Chem.*, 1929, **82**, 505.

[3] Jones, J. H., *J. Biol. Chem.*, 1926, **70**, 647.

[4] Brougher, J. C., *Am. J. Physiol.*, 1928, **84**, 583.

[5] Urechia, C. I., and Popovicius, G., *Compt. rend. Soc. de biol.*, 1928, **98**, 405.

[6] Shelling, D. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 301.

* See footnote † in Paper No. 5279.

† Dosage throughout these experiments is given as percent of the diet and so calculated as if cod liver oil of equal antirachitic potency were used.

peared. On the 8th day the serum calcium fell to 5.1 mg. % and tetany was severe. 1.0 mg. $CaCO_3$ and 500% viosterol were then added to the diet and the animal killed after 82 days. The animal was free of tetany and the serum calcium at the time was 9.1 mg. %.

3. Animals on stock diet in which 100% viosterol was incorporated lost the tetanic symptoms quite readily and after 60 days the serum calcium concentration was 10.0 mg. %.

4. On a high calcium, optimal* phosphorus diet plus 1000% viosterol, the serum calcium ranged between 8.5 and 12.7 mg. %.

5. With a low calcium, high phosphorus diet, and 1000% viosterol the serum calcium concentration was 9.2 mg. % in 60 days and tetany disappeared.

6. When 1% $CaCO_3$ was added to the above high phosphorus diet, the serum calcium was 10.0 mg. % in the same length of time.

Thus, in the absence of the calcium regulatory apparatus, the parathyroid gland, the shift of the calcium from the blood is so affected that ordinary dosages of viosterol, which are efficacious in normal animals, only partly or slightly affect the serum calcium levels of the organism deprived of its parathyroids. With larger doses and the removal of the additional strain, that of a deficiency in calcium in the diet, viosterol is capable of restoring the normal serum calcium level even after parathyroidectomy, provided treatment is continued for some time. It is worthy of note that the period of administration of viosterol in Greenwald's experiment lasted only 4 to 5 days—a period too short to expect an effect in an animal handicapped by thyroparathyroidectomy and by a calcium deficiency in the diet.

Recently Hess[7] also reports that he was able to raise the serum calcium level in thyroparathyroidectomized dogs and monkeys, if the previously reported large dose of viosterol was increased many times.

These experiments would seem to indicate the independent activity of viosterol. While in a normal animal it may act through and its activity be regulated by the parathyroid glands, in the absence of these glands, it may raise the serum calcium level to normal in the presence or absence of a calcium deficiency in the diet, provided large doses are used.

[7] Hess, A. F., Weinstock, M., and Rivkin, H., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 298.