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Summary. (1) Normal serum tocopherol values determined by a new photocolormetric method are given. (2) Untreated patients with amyotrophic lateral sclerosis show values within the normal range. (3) The blood level invariably rises on oral administration, irrespective of whether the patient does or does not show clinical improvement. (4) Intramuscular administration without simultaneous oral administration produces a drop in serum tocopherol.

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Comparison of Effects of Large Doses of Various Activated Sterols on Serum Calcium.

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When highly potent preparations of vitamin D became available, there was an immediate interest in the physiological effects of massive doses of the vitamin, and numerous studies dealing with their effects on the metabolism of calcium and phosphorus were reported. The literature has been reviewed by Schmidt and Greenberg,¹ and more recently by Reed, Struck and Steck,² therefore requires no extensive comment here. In general, it has been established that in normal animals large doses of vitamin D₂ result in a decreased fecal excretion of calcium, a hypercalcemia, and an increase in urinary calcium and phosphorus.

Other activated sterols might be expected to exert similar effects. Among these are vitamin D₃, dihydrotachysterol (A.T.10), and "Ertron". The last named is a form of activated ergosterol produced by the Whittier process and claimed by its manufacturers* not to have the hypercalcemic or other toxic effects of calciferol.³

¹ Schmidt, C. L. A., and Greenberg, D. M., *Physiol. Rev.*, 1935, **15**, 297.

² Reed, C. I., Struck, H. C., and Steck, I. E., *Vitamin D: Chemistry, Physiology, Pharmacology, Pathology, Experimental and Clinical Investigations*, Univ. of Chicago Press, 1939, p. 219.

* Nutrition Research Laboratories, Chicago, Ill.

³ Cf. also: Snyder, R. G., and Squires, W. H., *N. Y. State J. Med.*, 1940, **40**, 708.

Vitamin D₃ and A.T.10 have not been studied extensively experimentally probably because they have not been generally available. The literature on these preparations up to 1939 is included in the review of Reed, Struck and Steck. More recently Weber and Richardson⁴ have shown that in human subjects A.T.10 increases serum calcium and increases the absorption of calcium from the gut. Shohl and Farber⁵ have shown that A.T.10 exerts a hypercalcemic effect in rats considerably greater than would be predicted from its antirachitic value.

It is our purpose in this paper to present a comparative study of the effects on serum calcium of various amounts of the activated sterols mentioned, the doses being chosen in such a way as to remain within physiological limits. For test animals we selected adult albino rats from our laboratory stock colony; they varied from 175 to 240 g, and 90 to 120 days of age. They received, *ad libitum*, the regular breeding diet and water. The various preparations were administered by stomach tube as a single dose in 0.5 cc of peanut oil, and the animals were sacrificed by decapitation at the indicated intervals after medication. The blood was collected in centrifuge tubes, allowed to clot, and then centrifuged. Serum calcium was determined on each animal by the Clark and Collip⁶ modification of the Kramer and Tisdall⁷ method. The test groups consisted of at least 7 animals, and in some cases 10. A group of 34 control animals run at various times during the tests gave the average value for serum calcium of $11.15 \pm 0.35^{\dagger}$ mg %. Schmidt and Greenberg⁸ have reported this value as $11.3 \pm 0.50^{\dagger}$ mg %.

In one experiment doses of 25,000 I.U. of vitamins D₂, D₃ and of Ertron were given, the animals being sacrificed in groups at daily intervals up to 4 days. Since this treatment did not result in a hypercalcemia of the degree desired, we carried out a second experiment in which the doses were increased to 100,000 I.U. of each preparation. In this experiment we also gave 0.5 cc of commercial A.T.10 (0.5% solution), and 0.5 cc of a special A.T.10 (1.0% solution). Groups of animals were sacrificed at regular intervals as long as a significant hypercalcemia persisted. The results of the experiments are shown in Figs. 1 and 2.

⁴ Weber, F. C., and Richardson, H. B., *J. Clin. Endocrinol.*, 1941, **1**, 32.

⁵ Shohl, A. T., and Farber, S., *J. Nutrition*, 1941, **21**, 147.

⁶ Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

⁷ Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **47**, 475.

[†] Standard deviation.

⁸ Schmidt, C. L. A., and Greenberg, D. M., ref. 1, p. 334.

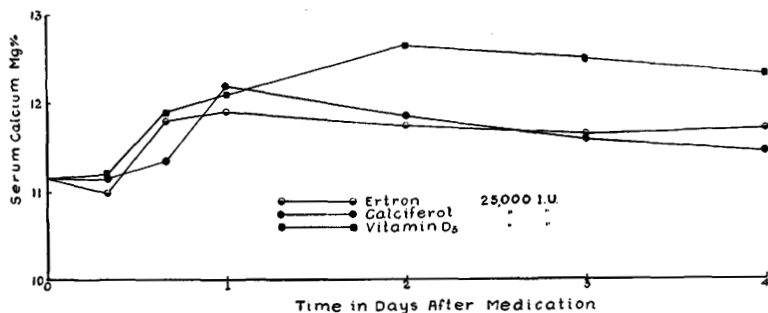


FIG. 1.

Effect of moderate doses (as to hypercalcemia) of vitamin D preparations on the serum calcium of the rat.†

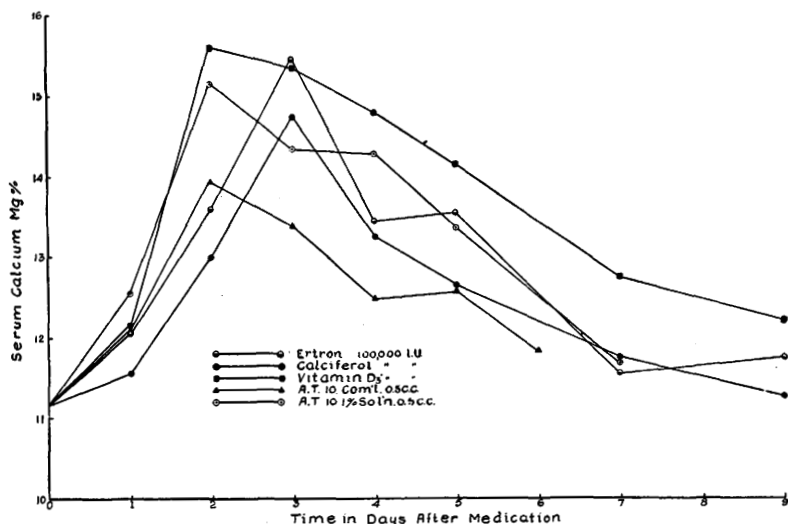


FIG. 2.

Effect of massive doses (as to hypercalcemia) of vitamin D preparations and of A.T.10 on the serum calcium of the rat.

Discussion. The most important conclusion which can be drawn from these experiments is that the effect of vitamin D₃ on serum calcium differs both quantitatively and qualitatively as to the type of curve produced from those of either of the activated ergosterol preparations. In other words, the serum calcium peak value following vitamin D₃ is not only reached at a different time, but the hypercalcemia induced persists for a significantly longer period of time than after activated ergosterol. Thus, the mass effect of vitamin D₃ (*i. e.*, elevation of serum calcium $\times$ time) is markedly greater (at least 50% on the 100,000 unit dose) than that of D₂.

† In both Fig. 1 and 2 each point represents the average of at least 7 animals.

A second conclusion is that Ertron and calciferol are indistinguishable in their effects on the serum calcium of the rat when corresponding U.S.P. or international antirachitic vitamin D dosages are administered. In both experiments the curves for the two products are not separated by a statistically significant amount at any point; the probable error calculated for each point is from 0.2 to 0.5 mg %.

The effects of the larger dosage of A.T.10 very closely resemble those of the 100,000 I.U. dosage of vitamin D₃. In terms of antirachitic activity, *i. e.*, vitamin D, the dose of this solution given represents only 22 to 25 I.U. per mg or a total dose of 110 to 125 units. Such a dosage is thus approximately 1/850 of the potency of the vitamin D₃ so that the effects of A.T.10 on serum calcium cannot be attributed primarily to the presence of contaminating antirachitic sterols. Finally, the marked difference between the effects of the two doses of A.T.10 given suggest that the determination of serum calcium might prove to be a satisfactory basis for the bioassay of the product to replace the original "toxic borderline dose" method.

Summary. The effects of vitamins D₂, D₃, and of Ertron and A.T.10 on serum calcium have been compared. Crystalline vitamin D₂ and Ertron are statistically indistinguishable in their effects. Vitamin D₃ produces a more prolonged hypercalcemia than does vitamin D₂. On the basis of responses to the above preparations, A.T.10 produces an effect far greater than would be predicted from its antirachitic value.

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Inhibition of Bacteriostatic Action of Sulfanilamide by Yeast Extracts.

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Substances capable of preventing the bacteriostatic action of sulfanilamide have been found widely distributed in nature.¹ Woods² reported that a substance was present in an extract prepared

¹ MacLeod, C. M., *J. Exp. Med.*, 1940, **72**, 217.

² Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.