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Chronic Toxicity and Hypercalcemic Effects of Various Activated Sterols in the Albino Rat.

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Toxicity studies on the various activated sterols have generally been carried out on white mice according to the technic of Holtz, Laqueur, Kreitmair, and Moll:[1] a determination of the so-called "toxic borderline dose" or "T.B.D." Data available in this laboratory indicate that the T.B.D.'s of vitamins D_2, D_3, and dihydrotachysterol are, respectively, 50 γ, 80 γ, and 6 γ, based on the weights of the crystalline substances.* The values reported in the literature have been tabulated by McLean,[2] and are of the same general magnitude.

Recently Mrazek, Novak, and Reed[3] reported a study of the chronic toxicity of vitamins D_2 and D_3 for the albino rat. Dosages of 75 I.U. per g per day were given to selected groups of 9 rats until all had died. They found that the average survival time of rats treated with vitamin D_2 was 43 days, and with vitamin D_3, 22 days. Thus it appears that vitamin D_3 is more toxic for the rat than vitamin D_2, but as pointed out above, less toxic than D_2 for the mouse. Morgan, Shimotori, and Hendricks[4] also concluded that vitamin D_3 is more toxic for rats than D_2, but Jung[5] concluded that the toxicities were identical.

The purpose of this study has been to compare, in the albino rat, the toxic and hypercalcemic effects of crystalline vitamins D_2 and D_3 and of commercial dihydrotachysterol with the object of determining whether there is any causal relationship between the two effects. This point cannot be regarded as settled at the present. It is the belief of Reed,[6] for example, that the toxic and hypercalcemic effects of vitamin D are not causally related. The hypercalcemic effects of single doses of the preparations named have been studied by McChesney and Kocher,[7] but data indicating their relative effects on continued administration to the rat are not available.

Experimental Methods. Albino rats of both sexes from our regular breeding colony served as experimental subjects. They ranged in age from 225 to 275 days at the start of the experiments, and weighed from 300 to 600 g. They were given our regular breeding diet[8] (Analysis: Ca, 0.49%; P, 0.53%) and water, *ad libitum*. The medications were given by stomach tube in corn oil, and each rat received 0.125 cc per 100 g body weight per day. Medications were not given on Sundays, therefore 0.1875 cc per 100 g was given on Saturdays and Mondays. (The concentrations of the solutions used ranged from 0.8-2.4 mg/cc.) The rats were weighed twice weekly and the doses were adjusted to these weights. Medications were continued until the animals died. The results of the experiments on chronic toxicity are given in Table I.

In order to determine the hypercalcemic effects of the activated sterols when administered daily, the same procedures were used. The animals were sacrificed by decapitation after 14 days of treatment since it was at

[1] Holtz, F., Laqueur, F., Kreitmair, H., and Moll, T., *Biochem. Z.*, 1931, **237**, 247.

* The commercial solution of dihydrotachysterol (Hytakerol) contains about 1250 γ of the active principle per cc. Hytakerol is a registered trademark of the Winthrop Chemical Co.

[2] McLean, F. C., *J. A. M. A.*, 1941, **117**, 609.

[3] Mrazek, R., Novak, C. R., and Reed, C. I., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 49.

[4] Morgan, A. F., Shimotori, N., and Hendricks, J. B., *J. Biol. Chem.*, 1940, **134**, 761.

[5] Jung, A., *Schweiz. med. Wochschr.*, 1943, **73**, 17.

[6] 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. 152.

[7] McChesney, E. W., and Kocher, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 156.

[8] Climenko, D. R., and McChesney, E. W., *ibid.*, 1942, **51**, 157.

TABLE I.
Survival of Albino Rats Receiving Various Activated Sterols Daily Until Death.

Preparation	Experiment	No. of rats	Dose, mg/kg/day	Survival time, days: Range	Survival time, days: Avg (median)	Equivalent toxic dose*
Vitamin D_2	A§	12	2.0	13-87	33 ± 5‡ (22)	
" "	C	12	2.4	16-72	43 ± 3.3 (42)	3.60
" "	D	10	3.0	11-82	33 ± 5.6 (25)	
" D_3	A	12	2.0	9-32	18 ± 1.5 (17)	
" "	B	8	1.5	19-124	59 ± 10 (44)	
" "	B	8	2.0	16-43	29 ± 2.5 (28)	2.30
" "	C	11	1.75	12-70	39 ± 4.3 (34)	
" "	D	10	2.25	7-74	27 ± 4.7 (20)	
Dihydrotachysterol	A	12	1.0	8-181†	34 ± 10 (18)	
"	C	12	1.12	9-67	24 ± 2.6 (20)	1.00
"	D	10	1.25	9-35	15 ± 5.4 (12)	

* Mg/kg/day—permitting a median 20-day survival time, as estimated from the data of Experiments A, C, and D.

† Animal sacrificed on the 181st day and serum Ca found to be 14.3 mg%. Judging from its condition the animal might still have survived for a considerable period of time.

‡ Probable error of the mean.

§ The experiment numbers are given in order to indicate which animals were run at the same time.

TABLE II.
Final Serum Calcium Values of Albino Rats Medicated Daily with Various Activated Sterols for 14 Days.

Preparation	No. of rats	Dose, mg/kg/day	Serum calcium, mg%: Range	Serum calcium, mg%: Avg	Dose required to produce serum Ca 15.3 mg%‡
Vitamin D_2	8	2.0	13.1-17.3	15.74 ± 0.38*	1.85
" D_3	8	1.0	13.5-15.4	14.61 ± 0.23	1.2
" "	8	1.5	15.3-17.1	16.30 ± 0.16	
Dihydrotachysterol	8	1.0	14.2-17.0	15.29 ± 0.24	1.0

* Probable error of the mean.

† Estimated from the data, as mg/kg/day for 14 days, assuming that the rise above the normal level of 11.2 mg% is proportional to the log of the dose.[12]

about this time that deaths generally began to occur in the chronic toxicity experiments. Blood was collected from each animal in a separate centrifuge tube, and calcium was determined on the serum by the method of Clark and Collip.[9] The results are given in Table II.

Discussion. The results as to the toxicity of vitamins D_2 and D_3 for the rat are in good agreement with the work of Mrazek, Novak and Reed, considering that slightly higher doses were used in this work. It is further established that dihydrotachysterol, on a weight basis, is more toxic than either vitamin

[9] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

[12] McChesney, E. W., and Kocher, H., *Endocrinology*, 1942, **30**, 787.

D_2 or D_3.

The toxicities of the 3 preparations do not completely correlate with their ability to elevate serum calcium. In the case of vitamins D_2 and D_3 the correlation is as satisfactory as can be expected from the experimental procedures: an equivalent toxic dose of vitamin D_2 is 57% greater, and an equivalent hypercalcemic dose 54% greater than that of vitamin D_3. However, if the toxicity of dihydrotachysterol depended on its hypercalcemic effect alone, one would expect the equivalent toxic dose (as determined experimentally) to be about 1.6 mg/kg/day instead of 1 mg/kg/day. This might have been anticipated since dihydrotachysterol as marketed is a mixture and its physiological action may be the summation of the effects of several substances.[10] It is difficult to conceive of crystalline materials such as vitamins D_2 and D_3 containing a hypercalcemic and an antirachitic principle distinct from each other. Correll and Wise,[11] using vitamin D from cod liver oil, were able to produce toxic effects in chicks without raising the blood calcium. On the other hand, toxic doses of irradiated ergosterol and of dihydrotachysterol did elevate the serum calcium.

The survival times reported must be considered as approximate since individual animals or groups of animals are evidently rather variable in their responses from time to time, due to factors beyond control. For example, in 2 experiments with vitamin D_2 the average survival times were 33 and 42 days although in the latter case the daily dose was 20% larger. Attention is also called to the fact that a small decrease in dose level may result in a disproportionate lengthening of the survival time. Thus, in Experiment B a decrease of 25% in the daily dose more than doubled the survival time. If the dose is not sufficiently large to kill the animals within the first three weeks, they appear to develop a tolerance which may permit them to survive for a considerable period of time. For this reason it is felt that the median survival times may be more significant than the averages.

Summary. For the albino rat, medicated daily until death, crystalline vitamin D_3 is about 55%, and commercial dihydrotachysterol about 260% more toxic than crystalline vitamin D_2 (based on the actual weights of the active principles). So far as vitamins D_2 and D_3 are concerned, the toxicities correlate very well with the hypercalcemic effects of the preparations. However, in the case of dihydrotachysterol the toxicity and ability to elevate serum calcium are not in that same proportion. In view of the difference in response of different species, these considerations do not necessarily apply to any species other than the albino rat.

[10] Werder, F. von, *Z. physiol. Chem.*, 1939, **260**, 119.

[11] Correll, J. T., and Wise, E. C., *J. Nutrition*, 1943, **26**, 641.

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Neuromuscular Transmission in Parathyroid Tetany.*

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Calcium is known to be an important factor in the transmission of nerve impulses. It has been shown that in its absence there is a failure of the liberation of acetylcholine at the synapse which otherwise occurs on stimulation of the nerve.[1] In view of this it was considered interesting to study neuromuscular transmission during hypocalcemia produced

* This work was performed with the aid of a grant from the Rockefeller Foundation.

[1] Harvey, A. M., and MacIntosh, F. C., *J. Physiol.*, 1939-40, **97**, 408.