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Comparison of Hypercalcemic Effects of Activated Sterols in the Chick.

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It was first demonstrated by Hess and Supplee[1] that irradiated ergosterol is much less effective, rat unit for rat unit, than cod liver oil in the prevention of rickets in the chick. The number of units of calciferol required to produce the same antirachitic effect as one U.S.P. unit of cod liver oil has been variously reported anywhere from 20 to 400, usually 30 to 50. The literature on this point has been reviewed by Remp and Marshall.[2] This phenomenon has never been satisfactorily explained, although the experiments of Russell and co-workers[3-5] have shed considerable light on the question. They have shown, for example, that the phenomenon is not attributable entirely to differences in absorption or to the route of administration. Further studies on the subject are in preparation for publication.[6]

The explanation of the remarkable difference of behavior between vitamins D_2 and D_3 in the chick must ultimately be referable to one or more of 3 factors: (1) a difference in the extent of absorption from the digestive tract, (2) a difference in the extent of destruction in the digestive tract, or rate of destruction in the tissues, or (3) a specific difference in the manner in which these substances affect calcium and phosphorus metabolism or the laying down of bone. In order to decide among these possibilities we have considered it advisable to review experimentally some of the previous work, and to add further experimental data.

It has been felt that considerable information about the problem might be gained by a determination of the relative amounts of the activated sterols required to produce an equivalent hypercalcemic effect in the chick. One would not expect the massive doses required to produce a definite degree of hypercalcemia to be subject to the same differences in absorption or the same extent of destruction as would apply to the relatively small doses needed to prevent rickets.

A study of the hypercalcemic effect of dihydrotachysterol has been included in this report since Correll and Wise[7] have shown that this substance, rat unit for rat unit, is about 5 times as effective in preventing rickets in the chick as is vitamin D from cod liver oil.

Methods. Male white leghorn chicks were raised from the second day of life on our modified diet of Hart, Kline, and Keenan (described by Remp and Marshall[2]) including 0.5% of cod liver oil to supply vitamin D. When the chicks were about 5 weeks old and averaged about 180 g in weight, they were divided into groups of 16 birds having as nearly as possible equal average weights. Each chick then received per 100 g body weight, 0.25 cc of the vitamin medication in corn oil (one dose only by stomach tube). On the second, third, fourth, and fifth days thereafter 4 chicks from each group were selected for sacrifice so that they would have the average weight of the entire surviving group. These 4 chicks were killed by decapitation, the blood was pooled for each group, centrifuged, and calcium was determined on the serum by the method of Clark and Collip.[8] Weight records were kept but are not included in the data since in only one experiment did the chicks lose weight and this occurred on all of the

1 Hess, A. F., and Supplee, G. C., PROC. SOC. EXP. BIOL. AND MED., 1929-1930, **27**, 609.

2 Remp, D. G., and Marshall, I. H., *J. Nutrition*, 1938, **15**, 525.

3 Klein, D., and Russell, W. C., *J. Biol. Chem.*, 1931, **93**, 693.

4 Russell, W. C., Taylor, M. W., and Wilcox, D. E., *ibid.*, 1932, **99**, 109.

5 Russell, W. C., Taylor, M. W., and Wilcox, D. E., *ibid.*, 1934, **107**, 735.

6 McChesney, E. W., to appear in *J. Nutrition*.

7 Correll, J. T., and Wise, E. C., *J. Nutrition*, 1942, **22**, 217.

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

TABLE I.
Hypercalcemic Effects of Activated Sterols in the Chick.

Substance	Dose mg/cc	Dose mg/kg	Serum calcium after, hours 48	72	96	120
Vitamin D_2	4	10	10.3	10.4	10.9	10.7
" "	8	20	10.6	10.4	10.7	10.6
" "	32	80	10.9	11.5	11.8	10.8
" "	50	125	10.7	12.1	10.9	10.7
" D_3	2	5	10.5	10.8	11.7	10.5
" "	4	10	11.6	12.3	11.2	11.7
" "	6	15	12.2	11.6	11.9	13.3
" "	8	20	12.4	13.3	11.9	10.8
Dihydrotachysterol	4*	10*	11.2	10.7	10.4	10.7
"	8	20	10.9	11.6	11.7	10.7
"	16	40	10.9	13.5	12.4	13.0
"	25	62.5	11.8	11.7	13.4	11.6
Corn Oil	—	—	9.6	9.9	10.6	10.1
" "				10.2		10.6
" "				10.7		10.4

*Based on actual weight of the active material. Thus a 10% solution contains about 2.5% dihydrotachysterol, and the commercial 0.5% solution contains about 0.125% dihydrotachysterol.

preparations studied. In every other experiment all groups gained weight at the same rate as the negative controls, even on the most massive doses. The results are given in Table I.

Metabolism of Vitamin D. Four chicks of the group which received 125 mg of vitamin D_2 per kg were selected for this experiment. They were put in a separate cage, and the feces were collected. Forty-eight hours after medication the chicks were sacrificed and the entire carcasses (with the exception of a few of the larger feathers) were digested under a reflux with 1 cc of 20% alcoholic KOH per g of bird. The digest was cooled, filtered, and extracted continuously with ether until all non-saponifiable matter was removed. The ether extract was then shaken with water, dried over sodium sulfate, and evaporated. The residue was taken up in propylene glycol for bioassay. The feces were dried, ground, and prepared in identical manner for bioassay.

Four chicks of the group which received 15 mg per kg of vitamin D_3 were also treated as described above in order to study the metabolism of this substance. The results of the experiments follow:

Vitamin D_2. Total dose to 4 chicks, 3,140,000 units; found in carcasses, 393,000 units or 12%; found in feces, 1,526,000 units or 49%.

Vitamin D_3. Total dose to 4 chicks, 432,000 units; found in carcasses, 118,000 units or 27%; found in feces, 155,000 units or 36%.

Discussion and Conclusion. As to the metabolism of vitamin D_3, the results agree with those of Klein and Russell[3] who found about 34% of vitamin D administered as cod liver oil in the feces. However, the amount of vitamin D_2 we find in the feces is considerably greater than the 26.5% reported by them. In both cases they gave much smaller doses. Our results indicate that vitamin D_3 by the peroral route is about twice as well absorbed as vitamin D_2. It is evident that the difference in the hypercalcemic effect of the two vitamins must be chiefly referable to the specific manner in which they affect calcium and phosphorus metabolism, since after 2 days the D_2 chick carcasses still contained 393,000 units, or 90% as many units as the total original dose of vitamin D_3. In spite of this, only a very small hypercalcemic effect was obtained (12.1 mg% maximum) in this group. In the vitamin D_3 group, in which only about 118,000 units were found after 2 days medication, a considerably higher maximum of 13.3 mg% was found. Differences of absorption and/or destruction in the digestive tract evidently cannot alone account for the very high doses of vitamin D_2 needed to produce the hypercalcemic effect.

It required 125 mg per kg of vitamin D_2 to raise the serum calcium to a level of 12 mg%. This level was also reached after a dose of 10 mg per kg of vitamin D_3, but not after a dose of 5 mg per kg. The ratio of effectiveness in producing hypercalcemia may therefore be set as approximately $D_3 : D_2 = 125 : 7.5$ or $16 : 1$. This ratio is significantly different from the generally accepted ratio of antirachitic effectiveness of 30 or 40 to 1, although we must consider the fact that the nature of these experiments does not permit an evaluation of better than $\pm 25\%$.

Dihydrotachysterol is surprisingly ineffective in elevating serum calcium in the chick. It required 40 mg per kg to produce a level of 13 mg%. This is, on a weight basis, about 3500 times the usual human daily dose.

The ratios of quantities of the activated sterols required to produce equivalent hypercalcemic effects (as to peak values) in the chicken contrast markedly with those required by the dog, which are:[9] D_2, 5 mg per kg; D_3, 5 mg per kg; and dihydrotachysterol, about 0.8 mg per kg. They also contrast markedly with those required by the albino rat, which are:[10] D_2, 12.5 mg per kg; D_3, 12.5 mg per kg; and dihydrotachysterol 6.25 mg per kg. These figures are not intended to be those which would produce the same peak value of 12 mg% in all species, but are simply those which produced the same peak in that species, whatever it might be, under the experimental conditions.

9 McChesney, E. W., and Messer, F., *Am. J. Physiol.*, 1942, **135**, 577.

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

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Physarum as a Radiation Test Object.

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One of the problems in experimental radiation has been to find a satisfactory test object which is on the one hand responsive to radiation and on the other hand sufficiently simply organized to make possible an analysis of the changes which occur. *Physarum polycephalum* partially fulfills this need. This slime mold is readily cultivated, macroscopically visible, rapidly growing, and continual streaming of its protoplasm gives an index of the direction in which it is growing as well as of the vigor of its growth in any given direction. In addition, we have found it to be sensitive to certain types of radiation.

Method. The plasmodium of *Physarum polycephalum* was grown in glass refrigerator dishes on paper towels sprinkled with oatmeal. The towels were kept just above the bottom of the dishes by pieces of glass, and the bottom of the container was covered with tap water. If the plasmodium is kept at a temperature below 20°C, there is little tendency to form fruit bodies; if the temperature falls below about 12°C, the cultures grow only slowly.

For testing, pieces of paper towel covered with plasmodium were transferred to one end of a refrigerator dish in the bottom of which there was an agar medium which was thin, flat, and translucent. A sheet of paper ruled to square millimeters was placed under this dish so that at any time the extent of the growth of the plasmodium could be accurately recorded by tracing the frontal edge on a similarly lined piece of paper.

In some cases many of the indentations were left out of the tracing if they were not important as it took too much time to go into such detail with a rapidly shifting contour. The radiation tubes were placed on the agar a short distance in front of the growth where it was growing best and fastest. This method of tracing has the disadvantage that it does