CLI. THE MINIMUM AMOUNT OF VITAMIN D REQUIRED FOR A POSITIVE ANTIRACHITIC EFFECT IN THE "LINE" TEST.

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FOSBINDER, Daniells and Steenbock [1928] made an attempt to determine quantitatively the amount of energy necessary to secure a demonstrable deposition of calcium in the bones of a rachitic rat. Using a monochromator and taking infinite pains to measure the energy transmitted from the radiation of $265\mu\mu$ wave-length from a mercury vapour lamp to a "pure" sample of cholesterol, they found that the shortest time of this treatment needed to produce enough vitamin D to give what they term a positive antirachitic action in their "line" test, was 22.5 seconds. The energy absorbed by the "cholesterol" during this time was 234 ergs. The number of quanta absorbed was calculated as 3.2×10^{13} . Applying Einstein's law of photochemistry, this would imply that 3.2×10^{13} molecules of vitamin D had been synthesised during the exposure of 22.5 seconds. The number of gram-molecules is then 5×10^{-11} and assuming the molecular weight of the antirachitic material to be essentially the same as that of cholesterol (385) the weight of vitamin D generated in this experiment was $5 \times 10^{-11} \times 385 = 2 \times 10^{-8}$ g. (It should be noted that this was the whole dose during the 10 days' test period, not a daily dose.) The American workers then prophesied that when pure vitamin D was obtainable this would be found to be approximately the amount required to give a positive result in the "line" test for the antirachitic factor. The writer has obtained results with a sample of irradiated ergosterol which appear to prove this prophecy to have been singularly correct.

The solution of irradiated ergosterol used in the Society's laboratory as a standard preparation of vitamin D was prepared by Mr T. A. Webster at the National Institute for Medical Research under conditions calculated to generate the maximum antirachitic activity in it. Its potency was determined by testing on rats rendered rachitic by the method of Steenbock and Black [1925]. The writer had had the privilege of 10 months' experience of this test under Prof. Steenbock's tuition, so that her standards and assays may fairly be considered comparable with his.

The result of the assay may be summarised thus:

Daily dose of irradiated ergosterol mg.	Degree of calcification in Steenbock's terms of assay
0.0	—
0.000005	Few foci
0.00001	+ (a thin line)
0.00002	++ (a thicker line)
0.00005	+++ (a thicker line still)
0.0001	++++ (complete healing)

Where Steenbock classified degrees of calcification as "negative," "positive," and "very positive," as he has done in his latest paper, his "positive" result may be considered equal to a + or ++ of the above classification, preferably the latter.

The daily dose that gave this positive result was 2×10^{-5} mg., so that the amount consumed in 10 days was 2×10^{-7} g.

Rosenheim and Webster [1927] state that maximum antirachitic activity is generated in ergosterol by their method of irradiation after 30 minutes and that at this time they can recover 90 % of unchanged ergosterol. Assuming that some of the vitamin D formed during the first 30 minutes of irradiation had been destroyed by the rays that appear to keep formation and destruction balanced during the subsequent 180 minutes, it would appear that rather less than 10 % of the irradiated ergosterol consisted of vitamin D. The amount of actual vitamin D contained in 2×10^{-7} g. of the Society's standard preparation of irradiated ergosterol would according to this be about 2×10^{-8} g. This gives an extraordinarily clear confirmation of the American workers' prophecy that the actual amount of vitamin D required to give a positive reaction in the "line" test would prove to be 2×10^{-8} g. Incidentally, also, there is evidence here of the suitability of the "line" test for demonstrating the presence of even smaller quantities than this of vitamin D.

REFERENCES.

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