X. THE QUANTITATIVE ESTIMATION OF VITAMIN D.

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IN a recent communication [Jephcott and Bacharach, 1926] we have described a method of detecting vitamin D by its effect on the faecal p_H of rats kept on a rachitogenic diet. In the curves shown in that paper there were indications of a quantitative relationship between the amount of vitamin and the extent of the change in p_H .

A series of doses of one batch of "ostelin" (an antirachitic extract of cod-liver oil prepared by a method based on that described by Zucker [1922]) has been fed to pairs of animals under conditions described in our previous communication. The results are shown in Fig. 1, curves *A* to *E*.

According to our custom, mixed samples of faeces were taken from groups of four animals until the beginning of the administration of the substance under test; the animals were then divided into two pairs, and the samples collected from these pairs. In the curves of Fig. 1 the preliminary period samples were collected together from groups *A* and *B* and from groups *C* and *D*; all the eight animals in those four groups were, it so happens, males from one litter. In the case of group *E*, the samples for the preliminary period represented those of the two animals used subsequently and two other animals used for a different test, all the animals in this case being females. The olive oil used for solution was entirely without effect on the faecal reaction of the animals, as has been found from the fact that tests of inactive substances dissolved in this oil have given quite negative results; the difference between curve *D* (four drops of olive oil) and curve *E* (one drop of olive oil) also bears this out.

The results of the experiments can be expressed in a slightly different manner. If the lowest value for the mean of two consecutive daily readings of faecal p_H ("the minimum mean p_H ") be plotted against the actual weight of vitamin-containing substance administered, a result is obtained which is shown in Fig. 2. Without wishing to draw too rigorous conclusions from a single series of observations on animals, however consistent, we think it justifiable to point out that the position of the values plotted suggests that they fall on an "S"-shaped curve, which is what might be expected from general considerations of the method of action of vitamin D. It would appear

Fig. 1. Curve A. 0.013 mg. "ostelin" dissolved in 22.5 mg. olive oil.
 Curve B. 0.026 mg. "ostelin" dissolved in 45 mg. olive oil.
 Curve C. 0.039 mg. "ostelin" dissolved in 67.5 mg. olive oil.
 Curve D. 0.053 mg. "ostelin" dissolved in 90 mg. olive oil.
 Curve E. 0.066 mg. "ostelin" dissolved in 22.5 mg. olive oil.

..... Basal diet only: no readings taken.
 - ● - - - Basal diet only: readings taken.
 —●— Basal and supplementary diets: readings taken.

that in this test the minimum amount of vitamin D which shows any appreciable effect is that which gives a lowering of faecal p_H to 7.1 and that the maximum effect is produced with an amount of vitamin D which gives a lowering of the faecal p_H to about 6.6. In our previous communication we wrote: "...until two consecutive readings give a mean value of p_H 6.7 or less, and neither more than 6.8. For our own laboratory convenience we have considered the quantity of antirachitic substance necessary for this purpose to be an antirachitic unit." Since then, we have found it convenient to subdivide this unit into ten. There are about 100 such units in a gram of the most active cod-liver oils.

Fig. 2.

SUMMARY.

Evidence is submitted that the extent of the lowering of faecal p_H by the administration of vitamin D to rats on a rachitogenic diet is a function of the amount of vitamin D administered.

REFERENCES.

- Jephcott and Bacharach (1926). *Biochem. J.* **20**, 1351.
Zucker (1922). *Proc. Soc. Exp. Biol. Med.* **20**, 136.