XXIV. THE DETERMINATION OF VITAMIN D.

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(Received December 30th, 1931.)

DYER [1931] has recently described a method of estimation of vitamin D based on the line test. He took 15 litters each consisting of 7 young rats and treated them as follows. Each litter was fed on a rachitogenic diet for 3 to 3½ weeks, and the different rats in the litter were then given different doses of a sample of irradiated ergosterol obtained from the National Institute for Medical Research. In each litter 1 rat received no irradiated ergosterol and the others received daily doses of $\frac{1}{16}$, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, 1 and 2 units vitamin D respectively. Dosage was continued for 10 days and then the ulnae and radii of the rats were examined by the line test. Dyer found that a given dose would produce different amounts of healing in different rats. However, by considering all 15 litters he was able to estimate the average healing produced by each dose and to express it in arbitrary figures. He then obtained a smooth curve relating average healing to dose of irradiated ergosterol given.

In similar tests Bills *et al.* [1931] estimated healing by giving one to four + signs to each animal. They examined a large number of rats and found the average healing produced by a given dose and by known multiples of that dose. From these results a curve was obtained relating potency of a solution to the average degree of healing produced.

Two experiments which involved the giving of graded doses of vitamin D to each of several litters of rats have recently been carried out in this laboratory. The results have been calculated in the same way as in Dyer's experiment and have been found to give curves relating the average degree of healing to the dose of vitamin D given. The curves are very similar to Dyer's curves and thus afford additional evidence of the validity of his method of estimating vitamin D.

EXPERIMENTAL.

1. *First experiment.* Ten litters of rats were used. One animal in each litter was given no vitamin D, 4 other animals were given daily doses of vitamin D of 0.25, 0.5, 1.0 and 2.0 units respectively. (The remainder of the animals in each litter were given special diets as described later in this paper.) The ulnae and radii were examined by the line tests and the degree of rickets shown by

each rat was estimated by two independent observers using the arbitrary scale proposed by Dyer. The values assigned to any one animal did not differ by more than 0.5 degree of healing. When this difference occurred, the average of the two values was determined. The results are given in Table I.

Table I.

Litter	Average value for degree of healing shown by rats receiving daily doses of vitamin D				
	No dose	0.25 unit	0.5 unit	1 unit	2 units
"Slight" rickets group.					
563	4	6	5.75	6	6
573	4.5	6	6	6	6
653	4	3	3.5	4.75	6
688	3	5.5	5.5	5	6
Average	3.9	5.1	5.2	5.4	6.0
"Severe" rickets group.					
583	0	3.25	4	6	6
589	0	3	5	4	4.75
620	0.5	4.5	5	5	6
622	0	4.5	4.25	5.5	6
644	0	5.5	4	4.25	6
700	0	2	4	5	5
Average	0.08	3.8	4.4	5.0	5.6

As in Dyer's experiment, the degree of rickets developed by each litter was judged to be "slight" or "severe". When healing was marked by a line, the width of the metaphyses could be seen in all the members of the litter, and the degree of rickets could therefore be judged by examination of all the rats of the litter. Thus the classification of the litter as having "slight" or "severe" rickets did not depend on the results given by the one or two animals which received no vitamin D. When the members of a litter only showed differences in the width of the metaphyses and no "lines" of healing, the grouping into "slight" or "severe" rickets was dependent wholly upon the value given by the rats which received no vitamin D.

Fig. 1. The relation between dose of vitamin D and the average degree of healing produced in groups of rats which had developed slight rickets.

It will be seen that the animals which received no vitamin D in the 4 litters 563, 573, 653, 688, developed a slight degree of rickets estimated as 3 or more

on the scale of healing, whereas those of the remaining 6 litters developed severe rickets. For each group, the dose of vitamin D was plotted against the average degree of healing produced by the dose. The "slight" rickets group gave curve *A*, Fig. 1, and the "severe" rickets group gave curve *B*, Fig. 2. Curves *D* and *D*₁ respectively are the corresponding curves given by Dyer. It is evident that these curves and Dyer's curves are very similar.

2. *Second experiment.* Litters of 8 or 9 individuals were chosen for this experiment. In each litter one or two animals were given no vitamin D and at least one animal received one of the following doses of vitamin D daily, 0.0625 unit, 0.125 unit, 0.25 unit, 0.5 unit, 1 unit, 2 units, 4 units. The results were estimated as before and the average values for the degree of healing are shown in Table II. As all these litters developed a severe degree of rickets, they were

Table II.

Litter	Average value for degree of healing shown by rats receiving daily doses of vitamin D							
	No dose	0.0625 unit	0.125 unit	0.25 unit	0.5 unit	1 unit	2 units	4 units
726	0.5	0	4.0	4.7	4.7	5.0	6.0	6.0
741	*0.5	2.8	2.3	4.3	3.5	3.5	4.3	6.0
737	0	1.2	1.2	4.2	4.2	4.8	6.0	*5.5
819	*0.6	0.7	1.8	1.8	2.5	4.2	4.8	5.2
833	0	0.7	1.0	2.2	3.8	4.3	5.2	6.0
818	0.2	0.8	1.3	1.7	3.2	4.5	3.2	4.8
Average	0.30	1.0	1.9	3.15	3.65	4.4	4.9	5.6

* Average of values from 2 rats.

Fig. 2. The relation between dose of vitamin D and the average degree of healing produced in groups of rats which had developed severe rickets.

averaged in one series only. The relation between the dose of vitamin D and the average degree of healing produced in the rats used in this experiment is shown in curve *C*, Fig. 2. Each point on this curve is given by the average of the values obtained from at least 6 individuals. Where 2 rats in a litter received the same dose, the two results were averaged to prevent overweighting of that litter in the average for the dose.

The curve for these litters is also similar to the curve obtained in the first

experiment and to Dyer's curves. As, however, they are not exactly alike, some uncertainty might be felt as to which curve should be used for comparison of two results in the way suggested by Dyer. An attempt has, therefore, been made to see how far a comparison is influenced by the choice of the curve of reference.

THE USE OF DIFFERENT CURVES FOR THE ESTIMATION OF THE
VITAMIN D POTENCY OF AN UNKNOWN SUBSTANCE.

In the method described by Dyer, the potency of an unknown substance may be compared with that of the standard solution by using either curve *D* or *D*₁, according to whether the degree of rickets shown by the animals receiving no vitamin D is less or more severe. The comparison was made by determining the average degree of healing for a given dose of unknown and for a given dose of standard vitamin D. The ratio of the corresponding abscissae on the appropriate curve gave the ratio of the vitamin D contents of the two doses.

Dyer gave an example in which the average value for the degree of healing shown by the animals receiving no dose in 3 litters was 2.8. The values for daily doses of 0.5 g. unknown sample *X* and 0.5 unit vitamin D were 4.7 and 5.1 respectively. Since these litters developed only moderately severe rickets, the abscissae corresponding to 4.7 and 5.1 degrees of healing were determined by reference to curve *D*. Hence

$$\frac{\text{Vitamin D content of 0.5 g. sample } X}{0.5 \text{ unit vitamin D}} = \frac{0.31}{0.525} = 0.59.$$

Curve *A* was compiled by a similar method and under similar conditions to curve *D*. It is not coincident with curve *D*, but almost parallel to it. This may be due to some unrecognised factor acting in one experiment and not in the other. Suppose the activity of sample *X* were determined by reference to curve *A*, then

$$\frac{\text{Vitamin D content of 0.5 g. sample } X}{0.5 \text{ unit vitamin D}} = \frac{0.17}{0.32} = 0.53.$$

Thus the potency of sample *X* would be given as 0.59 unit per g. using curve *D* and 0.53 unit per g. using curve *A*. The difference is only 12 % and this in spite of the fact that curve *A* was constructed from 4 litters only.

Table III. *Showing the use of different curves for determining the potency of an oil when 5 mg. oil gives 3.8 degrees of healing and 0.5 unit vitamin D gives 4.55 degrees of healing.*

Curve	$\frac{\text{Vitamin D content of 5 mg. oil}}{0.5 \text{ unit vitamin D}}$	Potency of oil in units per g.
<i>B</i>	$\frac{0.28}{0.54} = 0.52$	52
<i>C</i>	$\frac{0.50}{1.03} = 0.49$	49
<i>D</i>	$\frac{0.45}{0.87} = 0.52$	52

The curves *B*, *C* and *D*₁ obtained from litters which developed severe rickets may be similarly compared. For example, in a test of a sample of cod-liver oil, the average degree of healing shown by the animals which received no dose was 0.8. The values for 5 mg. cod-liver oil and 0.5 unit vitamin D were 3.8 and 4.55 respectively. The results given by the three curves are shown in Table III. The difference is only 6 %.

It will be seen that the same result is obtained from all three curves. This may be confirmed by taking pairs of imaginary results and evaluating them from each curve.

DISCUSSION.

The division into "slight" and "severe" rickets is necessarily arbitrary. Dyer included in the "severe" group only litters in which the animals receiving no vitamin D gave the value 1.5 or less. Presumably any litter in which the value was greater than this should be included in the "slight" group. In the compilation of curve *D*, however, the lowest value obtained for any rat receiving no dose was 2.6. We have found in practice that when the average value given by the animals receiving no vitamin D in a litter lies between 1.5 and 2.5, the corresponding value for the unknown or for a dose of 0.5 unit vitamin D is often below 3.4. According to Dyer's method, the curve for "severe" rickets is the only one available for the interpretation of such results, since values below 3.4 cannot be measured on curve *D*. All Dyer's results and the results from the experiment here described, were, therefore, collected together to form three curves representing the effect of different doses of vitamin D on litters which developed "slight," "moderate" and "severe" rickets respectively.

Fig. 3. Summary of all results described in this paper (including Dyer's results).

In Fig. 3, curve I represents the "slight" rickets group in which the value for the degree of healing shown by each animal receiving no vitamin D was greater than 3.0. Curve II represents the "moderate" rickets group in which the values for these animals ranged from 1.0 to 3.0 inclusive. Curve III represents the "severe" rickets group in which the values for these animals lie

below 1.0. For the compilation of these curves the average degree of healing was found for each dose represented in every litter in the group. Curve I was obtained from 10 litters, curve II from 4 litters and curve III from 17 litters. Curve II would be expected to lie between curves I and III as indicated by the continuous line in the diagram. The best curve through the experimental points is, however, that given by the dotted line. This difference is probably due to the fact that only 4 litters were used for the construction of curve II. All the curves are approximately logarithmic in shape.

COMPARISON OF THIS METHOD WITH A PROPHYLACTIC METHOD
(ASH CONTENT OF BONES) FOR TESTING FOR VITAMIN D.

A sample of fish meal was examined by the curative method described in this paper and also by a prophylactic method based on the percentage ash in the dry fat-free bones. Details of the two experiments are given below.

1. *Curative method.* The line test was carried out and the results evaluated in the usual way. Three litters of rats were used consisting of 3, 5 and 6 individuals respectively. They all developed severe rickets, the average degree of healing given by the animals which received no dose being 0.7. The average values for the rats which received 2.5 % fish meal in the diet and 0.25 unit vitamin D daily were 5.1 and 4.35 respectively. By reference to curve III (Fig. 3), the ratio $\frac{5.1}{4.35}$ for healing was found to correspond to the ratio $\frac{1.7}{0.7}$ for dose of vitamin D. Records of the total amount of food eaten daily by each rat receiving 2.5 % fish meal, showed that the average amount of fish meal eaten per rat per day was 0.22 g. Therefore

$$\frac{\text{Vitamin D content of 0.22 g. fish meal}}{0.25 \text{ unit vitamin D}} = \frac{1.7}{0.7} = 2.43,$$

1 g. fish meal, therefore, contains $\frac{2.43 \times 0.25}{0.22} = 2.75$ units vitamin D.

2. *Prophylactic method (ash content).* This experiment was based on the method described by Chick *et al.* [1926]. In each of the 5 litters used for the test some of the rats received no dose, others received 0.1 unit vitamin D daily and others received 0.5 % fish meal incorporated in their food. The basal diet for all the animals was Steenbock's rachitogenic diet as used in the curative test. The feeding was continued for 28 days, after which the animals were killed and their humeri and femora were removed and dried. The fat was then extracted from the bones with hot alcohol. The percentage ash in the dry fat-free bones was determined for each rat. The results are given in Table IV. The average values for the rats receiving 0.5 % fish meal and 0.1 unit vitamin D daily were 37.3 and 40.1 respectively. These values are not very different from each other but both are higher than the corresponding figure of 29.4 found for the rats which received no dose. The average amount of fish meal eaten per rat per day was 0.039 g. The results show, therefore, that 0.039 g. fish meal contains approximately 0.1 unit vitamin D. 1 g. fish meal,

Table IV.

Showing percentage ash in dry fat-free bones of rats receiving

Litter	No dose		0.5 % fish meal		0.1 unit vitamin D	
	Rat	% Ash	Rat	% Ash	Rat	% Ash
1199	6975	27.0	6977	41.1	6979	46.1
	6976	26.25	6978	37.3	6980	40.7
1193	6940	29.1	6942	32.3	6944	39.4
	6941	33.8	6943	40.5	6945	40.6
1208	7027	31.6	7029	38.0	7031	31.9
	7028	30.1	7030	40.7	7032	40.9
1213	7051	30.2	7052	37.3	7053	42.3
1218	7074	26.8	7075	31.0	7076	39.3
Average values		29.4		37.3		40.1

therefore, contains 2.5 units. Thus the result obtained by this method agrees closely with the value 2.75 units per g. given by the line test.

Since we have no satisfactory curve relating the percentage ash to dose of vitamin D, we cannot evaluate the results given by the prophylactic test unless the dose of unknown substance produces an effect similar to that of the dose of standard as in the example given above. Otherwise the test will only show whether a given dose is more potent or less potent than the dose of standard without indicating how much more, or less, potent it may be. The use of the curve for the interpretation of values, therefore, gives more precise results.

THE EFFECT OF ALTERING THE RATIO OF CALCIUM TO PHOSPHORUS IN THE BASAL DIET.

In the routine testing of the vitamin D content of foodstuffs, it has sometimes been found necessary to incorporate in the diet large quantities of the substance to be tested. It seemed possible that such substances might alter the ratio of calcium to phosphorus in the diet sufficiently to affect the degree of rickets produced in the rats. The rachitogenic property of Steenbock's diet is due to its high value for Ca/P together with its lack of vitamin D. Karelitz and Shohl [1927] showed that when they added to their diet sufficient sodium phosphate to change the ratio of calcium to phosphorus from 4.25 : 1 to 1 : 1 "definite healing" was brought about in 24 to 48 hours. In our routine tests the alteration in the relative amounts of calcium and phosphorus has always been less than this and an experiment was devised to find the effect of small changes in the ratio.

A representative sample of the rachitogenic diet in general use in these laboratories was ground to a fine homogeneous powder in a ball mill. Analysis showed it to contain 1.37 % calcium and 0.341 % phosphorus. The ratio of calcium to phosphorus was, therefore 4 : 1. This diet is referred to in this paper as "diet I." Diets II, III and IV were made with the ratio of calcium to phosphorus 4 : 2, 4 : 3 and 4 : 4 respectively. The ratio was adjusted by the addition of calcium phosphate with the removal of the necessary amount of calcium carbonate. The composition of the diets is given in Table V.

Table V. *Percentage composition of diets.*

	I* Ca : P=4 : 1	II Ca : P=4 : 2	III Ca : P=4 : 3	IV Ca : P=4 : 4
Yellow maize	76	76	76	76
Gluten flour	20	20	20	20
Sodium chloride	1	1	1	1
Calcium carbonate	3	2	0.85	—
Calcium phosphate	—	1.5	3.0	4.1

* Steenbock's rachitogenic diet No. 2965.

Ten litters of rats were prepared for the test in the usual way by being fed for 3 to 3½ weeks on the rachitogenic diet (No. I) in which the ratio of calcium to phosphorus was 4 : 1. In each litter one or two rats continued to receive this diet during the 10 days' test, 3 other rats received diets II, III and IV respectively, and the remaining animals received diet I together with daily doses of 0.25, 0.5, 1 and 2 units vitamin D respectively. The results were estimated in the usual way. The values obtained for the control animals are those given in Table I and summarised in the curves *A* and *B* in Figs. 1 and 2. Litter 688 was the only litter which might be included in the "moderate" group. The animal in this litter which received no vitamin D gave a value of three degrees of healing, a figure which is on the border line of our arbitrary division between "moderate" and "slight." For this investigation, therefore, the litters were divided into two groups only—those showing "slight" and those showing "severe" rickets. Litter 688 was included in the first group. The results are summarised in Table VI.

Table VI.

Litters	Degree of healing shown by rats which received							
	Diet I (Ca : P =4 : 1)	Diet II (Ca : P =4 : 2)	Diet III (Ca : P =4 : 3)	Diet IV (Ca : P =4 : 4)	Diet I + 0.25 unit vitamin D daily	Diet I 0.5 unit + vitamin D daily	Diet I + 1.0 unit vitamin D daily	Diet I + 2.0 units vitamin D daily
"Slight" rickets group.								
563	4.0	6.0	6.0	6.0	6.0	5.75	6.0	6.0
573	4.5	5.0	6.0	6.0	6.0	6.0	6.0	6.0
653	4.0	4.0	6.0	6.0	3.0	3.5	4.75	6.0
688	3.0	6.0	6.0	6.0	5.5	5.5	5.0	6.0
Average value	3.9	5.25	6.0	6.0	5.1	5.2	5.4	6.0
"Severe" rickets group.								
583	0	5.0	*5.5	4.75	3.25	4.0	6.0	6.0
589	0	4.0	4.5	4.75	3.0	5.0	4.0	4.75
620	0.5	4.25	6.0	5.75	4.5	5.0	5.0	6.0
622	0	6.0	6.0	6.0	4.5	4.25	5.5	6.0
644	0	4.75	6.0	6.0	5.5	4.0	4.25	6.0
700	0	5.0	6.0	6.0	2.0	4.0	5.0	5.0
Average value	0.08	4.8	5.7	5.5	3.8	4.4	5.0	5.6

* Average of values from two rats.

It will be seen that in the "slight" rickets group, diets III and IV both produced complete healing of rickets. In other members of the same litters a daily dose of 2 units of vitamin D given in addition to diet I also produced complete healing, whereas lower doses of the vitamin produced only partial healing. Decreasing the ratio of calcium to phosphorus from 4 : 1 to 4 : 3 is, therefore, equivalent to giving a daily dose of 2 units vitamin D. The "severe" rickets group shows a similar result. Diet III gave an average value of 5.7 degrees of healing and 2 units vitamin D gave an average value of 5.6 degrees of healing. These figures are too high to be found on the sensitive part of the curve for rats showing severe rickets, but they confirm the result given above that diet III is equivalent in effect to a daily dose of 2 units vitamin D. In the "severe" rickets group, diet IV, in which the ratio of calcium to phosphorus is approximately 1, gave a value for the average degree of healing slightly less than that given by diet III. This result may be due to the variation in animals or it may indicate that a calcium to phosphorus ratio of 4 : 3 is the optimum for curing severe rickets. Any further increase in the proportion of phosphorus would then hinder the cure and tend to produce the condition known to be brought about by diets in which the ratio P/Ca is high.

Further comparisons were made by referring the values for the degree of healing produced by diet II to curves *A* and *B*, which were drawn from the animals which had received graded doses of vitamin D in these particular litters. The corresponding abscissae gave the equivalent dose of vitamin. Thus in the "slight" rickets group, diet II produced 5.25 degrees of healing. Reference to curve *A*, Fig. 1, shows that 5.25 degrees of healing would be given by a daily dose of 0.40 unit vitamin D. Diet II is, therefore, equivalent in anti-rachitic effect to a daily dose of 0.40 unit vitamin D. In the "severe" rickets group, diet II produced 4.8 degrees of healing. Reference to curve *B*, Fig. 2, shows that this value would be given by a daily dose of 0.70 unit vitamin D. This result is higher than that obtained from the "slight" rickets group. In order to determine whether the difference was due entirely to experimental error, results from 5 more litters were examined. The members of these litters were grouped so that at least 1 rat in every litter received one of the following diets—diet I, diet II, diet III, diet IV, diet I + 0.5 unit vitamin D daily, diet I + 1.0 unit vitamin D daily. The rats receiving vitamin D in these litters were not originally included in curves *A* and *B* since some of the doses of vitamin D were not represented in every litter. The effect of the diets was determined by a direct application of Dyer's method for estimating the potency of any unknown substance. The results were referred to the general curves in Fig. 3. Full details of the degree of healing obtained with the diets and with daily doses of 0.5 unit and 1.0 unit vitamin D are given in Table VII. The average values for diets III and IV were too high for the calculation of their relative potencies by means of the curves. The values for diet II were, however, referred to curves I and III (Fig. 3) and the corresponding abscissae were compared with those obtained from 0.5 unit and 1.0 unit vitamin D. The results

Table VII.

Litter	Degree of healing shown by rats which received					
	Diet I (Ca : P = 4 : 1)	Diet II (Ca : P = 4 : 2)	Diet III (Ca : P = 4 : 3)	Diet IV (Ca : P = 4 : 4)	Diet I + 0.5 unit vitamin D daily	Diet I + 1 unit vitamin D daily
"Slight" rickets group.						
461	*4.25	*5.6	*6.0	*5.0	5.75	6.0
463	3.75	3.25	4.0	6.0	*4.6	*4.6
465	4.0	5.25	5.0	3.0	*2.0	*4.6
486	3.0	4.0	6.0	5.0	2.0	5.0
563	4.0	6.0	6.0	6.0	5.75	6.0
573	4.5	5.0	6.0	6.0	6.0	6.0
653	4.0	4.0	6.0	6.0	3.5	4.75
688	3.0	6.0	6.0	6.0	5.5	5.0
Average value	3.8	4.9	5.6	5.4	4.4	5.2
"Severe" rickets group.						
483	*0.5	4.9	*5.5	*5.9	5.25	6.0
583	0	5.0	*5.5	4.75	4.0	6.0
589	0	4.0	4.5	4.75	5.0	4.0
620	0.5	4.25	6.0	5.75	5.0	5.0
622	0	6.0	6.0	6.0	4.25	5.5
644	0	4.75	6.0	6.0	4.0	4.25
700	0	5.0	6.0	6.0	4.0	5.0
Average value	0.1	4.8	5.6	5.6	4.5	5.1

* Average of values from two rats.

Table VIII.

Dose with which diet II was compared	Ratio of degrees of healing on Diet II to dose of vitamin D	Ratio of corresponding abscissae obtained from curve	Daily dose of vitamin D equivalent to diet II
"Slight" rickets group.			
0.5 unit vitamin D	$\frac{4.89}{4.39}$	$\frac{0.28}{0.09} = 3.1$	1.5 units
1.0 unit vitamin D	$\frac{4.89}{5.24}$	$\frac{0.28}{0.54} = 0.52$	0.52 unit
"Severe" rickets group.			
0.5 unit vitamin D	$\frac{4.84}{4.50}$	$\frac{1.2}{0.82} = 1.5$	0.75 unit
1.0 unit vitamin D	$\frac{4.84}{5.11}$	$\frac{1.2}{1.63} = 0.74$	0.74 unit

are shown in Table VIII. In the "severe" rickets group, diet II was found to be equivalent to a daily dose of 0.75 or 0.74 unit vitamin D. These results agree closely with each other and confirm the value 0.70 unit found by direct reference to curve *B* as shown above.

In the "slight" rickets group, diet II was found to be equivalent to 1.5 units vitamin D when compared with one dose of standard and 0.5 unit when compared with the other dose of standard, whereas direct reference to curve *A* gave

the value 0.4 unit. These differences are very much greater than those obtained in the "severe" rickets group and appear to confirm the suggestion of Bourdillon *et al.* [1931] that the variation in response is greater for rats which develop slight rickets than for rats which develop more severe rickets. No reliable information could be gained from such widely different results and we have assumed that the most probable value for the daily dose of vitamin D equivalent to diet II is 0.7 unit, which is the value given by the "severe" rickets group.

This result is of importance in the testing of the vitamin D potency of a substance containing a high proportion of phosphorus. Suppose the unknown substance were incorporated in the diet in sufficient quantity to change the ratio of calcium to phosphorus from 4 : 1 to 2 : 1, then healing equal to that produced by 0.7 unit vitamin D would be due to the change in the ratio of calcium to phosphorus and only additional healing could be attributed to the vitamin D in the unknown substance. In the absence of any figures relating smaller alterations in the ratio Ca/P to equivalent doses of vitamin D, it may be assumed that the relationship between healing and Ca/P ratio is given by a curve somewhat similar to that relating healing and dose of vitamin D. It would then appear desirable that, if the ratio Ca/P is changed from 4 : 1 even to 4 : 1.25, the diet of the control animals should be adjusted so that the ratio is the same as in the diet containing the test substance.

SUMMARY.

Two experiments are described in which graded doses of vitamin D were given to young rats in two series of 10 and of 6 litters respectively. The degree of healing shown by each rat was assessed according to Dyer's scale and curves were drawn relating the average degree of healing to the dose of vitamin D. The curves were similar in shape to Dyer's curves and gave approximately the same results for the potency of unknown substances. The results from these experiments were combined with those of Dyer and divided into three groups as follows:

- (a) litters which developed "slight" rickets;
- (b) litters which developed "moderate" rickets;
- (c) litters which developed "severe" rickets.

A curve relating degree of healing to dose of vitamin D was drawn for each group.

The curves were used to estimate the vitamin D potency of a sample of fish meal and the results were found to agree with those obtained in a prophylactic test based on the percentage of ash in the dry fat-free bones. It was shown that the curative test is more generally useful than the prophylactic test since differences in degree of healing could be evaluated by reference to the curves.

The effect of changing the ratio of calcium to phosphorus in Steenbock's rachitogenic diet was determined by the curative test, using the curves to evaluate differences in the average degree of healing produced by different diets. It was found that changing the ratio from 4 : 1 to 2 : 1 produced an anti-rachitic effect equal to that given by a daily dose of 0.7 unit vitamin D.

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