CXXVI. THE ASSAY OF VITAMIN A.

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HUME and SMITH [1928] in a recent comprehensive survey of methods of assay of vitamin A have summarised the different kinds of response to different doses of vitamin A in animals previously depleted of this factor, as follows.

- (a) Very small or no dose. No recovery.
- (b) Small doses. Subnormal growth, graded quantitatively to the dose; later premature slackening.
- (c) Larger doses. Normal growth for a time followed by premature slackening.
- (d) Optimal dose. Normal growth to maturity.

Hume and Smith based this scheme partly on their own experience and partly on that of other workers. The writers have had this experience many times. But other types of response have also been encountered, a knowledge of which may eventually help in the elucidation of the rôle of the vitamin in the animal body. In tests on certain substances submitted to this laboratory for vitamin A assay, subnormal growth, graded quantitatively to the dose, has been obtained but instead of being followed by premature slackening the rate of growth has remained unaltered, and subnormal, for unusually long periods of time, some animals attaining maturity only after about 40 weeks on the experimental diet.

Another variation in response to equal doses of the same substance is also demonstrated. Animals which are apparently in a similar physiological or pathological condition, when considered ready for a curative test, have shown:

- (a) immediate resumption of rapid growth;
- (b) immediate resumption of subnormal growth;
- or (c) a long latent period, often of several weeks, followed by a resumption of normal growth.

Technique.

The technique of these experiments was that adopted throughout in these laboratories [Steenbock and Coward, 1927]. The sample of dried yeast used in the tests described in this paper was one which caused resumption of "normal" growth in a dose of 0.4 g. daily in a curative test on the vitamin B-free diet in use in this department. Hence its incorporation as 8 % of the vitamin A-free diet makes it certain that vitamin B was not a limiting factor in these experiments.

*Examples of the continuous, subnormal response.*1. *An assay of vitamin A in a sample of cod-liver oil.*

The result obtained in the first 80 days of the curative period was practically in accordance with Hume and Smith's scheme, but the experiment was continued for a further period of 190 days for another purpose which will be reported later. The rat on the lowest dose of oil, 0.001 g., lived for a further 63 days and then died. The other three rats, still given their respective doses, maintained very slow growth for a further 90 days. They were then given a daily dose of 0.5 g. marmite dissolved in water in order to ascertain whether a shortage of vitamin B had possibly been the limiting factor in their growth. When this had shown no influence on their rate of growth for 30 days, it was

Fig. 1. Curves of growth of rats given different doses of cod-liver oil. The growth was graded to the doses during the first 80 days, then continued more uniformly nearly to maturity, the rat on the lowest dose, only, dying.

discontinued and 4 days later the daily ration of cod-liver oil was in each case increased to 0.2 g. The two lighter rats, 167 and 168, previously receiving the smaller doses of cod-liver oil, 0.002 and 0.005 g. respectively, responded to the higher dose in a very marked manner, growing to about 140 g., at which weight growth of does of this strain (pure bred albinos) tends to fall off somewhat. The fourth rat which had received the highest dose of cod-liver oil throughout the test (0.01 g.) was nearly mature when the dose was increased and merely maintained its former rate of growth. It must be accepted therefore that these are vitamin A curves throughout; for the behaviour on the highest dose of cod-liver oil proved that the rats had received ample supplies of vitamin B. (The lack of response to the extra vitamin B in the marmite does not prove this as the low supplies of vitamin A were evidently the limiting factor to growth at this point (Fig. 1).)

After the first more rapid response, the rats settled down to subnormal, steady rates of growth for a further 17 weeks with no premature slackening.

2. *An assay of vitamin A in a certain biscuit.*

The biscuit, finely powdered, was incorporated in the diets of the rats as 5, 10, 20 and 40 % respectively. Daily records of food intake were made and from them was calculated the total intake of the biscuit eaten by each rat per week. These figures are given in Table I as they are more instructive than the figures representing the percentages of the mixture incorporated in the diets.

Table I.

Rat	% mixture in diet	Total intake of vitamin mixture per week (g.)								
		1	2	3	4	5	6	7	8	9
M 216	5	1.8	1.9	2.0	1.8	2.4	2.4	2.0	2.6	2.9
259	"	1.9	1.8	2.5	2.2	2.5	2.8	3.4	3.4	—
330	"	2.2	2.5	2.2	2.6	2.6	2.8	2.5	3.7	—
212	"	1.8	2.4	1.7	2.3	2.3	2.3	3.0	2.8	—
211	10	5.0	5.6	6.7	6.9	7.8	7.8	7.8	8.6	7.9
258	"	3.6	4.0	4.6	4.6	5.8	5.5	4.9	6.1	6.5
254	"	2.9	3.1	3.8	4.1	4.7	4.7	3.9	4.0	4.3
326	"	4.3	4.6	4.7	4.9	5.4	6.2	6.4	—	—
327	20	7.6	9.8	9.8	10.6	10.4	12.6	15.2	—	—
214	40	14.8	15.2	18.0	19.2	20.0	20.0	19.2	25.0	22.4

(Other rats used in this assay gave the more general type of recovery curve and therefore are not quoted here.)

The rates of growth of these rats are nearly constant throughout the time of the experiments. They are subnormal, graded to the dose, and do not show any sign of premature slackening (Fig. 2).

3. *An assay of vitamin A in the general London milk supply of November and December, 1927.*

Three bucks of one litter used in this test gave subnormal growth, graded to the dose, without premature slackening. The curves of growth are shown in Fig. 3.

4. *An assay of vitamin A in a certain mixture submitted to this department for analysis.*

Three bucks of the same litter as those in the last experiment were used in this test and again gave subnormal growth, graded to the dose, without premature slackening. The curves of growth are shown in Fig. 4. The mixture was incorporated in the diets as 2, 5 and 20 % respectively. Daily records of food intake were made and the amounts of the mixture eaten per week were calculated. The figures are summarised below.

Rat	% mixture in diet	Total amount of mixture eaten per rat per week (g.)						
		1	2	3	4	5	6	7
G.R.L. 32 A	2	0.92	0.94	0.94	0.94	0.94	1.4	1.4
" C	5	2.0	1.95	1.95	2.1	2.1	2.25	2.5
" E	20	9.0	9.6	9.8	9.2	11.4	11.6	14.8

Varied types of response to the same dose.

Three different types of response to a given dose of a substance containing vitamin A are frequently met. They may be described as (a) immediate resumption of rapid growth which may be continued to maturity, (b)

Fig. 2. Growth of rats, graded to the dose of vitamin A, without premature slackening. A more accurate comparison is obtained by comparing these curves with the weekly records of intake of the vitamin mixture rather than with the percentage incorporated in the diet.

Fig. 3. Growth of rats, graded to the dose of London milk. No premature slackening.

Fig. 4. Growth of rats, graded to the dose of vitamin A. The weekly intake corresponds with the percentage of the material in the diet in this experiment.

immediate resumption of growth but at a lower rate than (a), (c) a long steady period followed by rapid resumption of growth. In the last, presumably, growth is suspended until possible pathological conditions are cleared up. Curves showing the variations in response to a given dose are given in Fig. 5. The variability of the response is discussed here because it is obviously of the greatest importance to give a rat the chance of recovering from disorders before deciding that a particular dose of a substance is inadequate in vitamin A. It must be admitted, however, that this cannot be the whole explanation, for far more uniform and comparable results are obtained when rats from one litter are used for a comparison than when a miscellaneous selection of unknown history are used, even though, from a consideration of their growth curves, they may appear to be in a similar condition.

Fig. 5. Two examples of variation in response to equal doses of the same substance. Rats M 386, 467 show immediate resumption of rapid growth; rats 335 and 346 show immediate resumption of subnormal growth; rats 334 and 345 show a long latent period followed by resumption of rapid growth.

For an assay of the vitamin A content of a substance in this laboratory three or four rats are used for testing each of several different doses, the lowest of which is expected to be too small for resumption of growth, and the highest probably greater than is necessary for normal growth. A comparison of the whole series of results with those obtained from a similar series on the substance with which the unknown is to be compared, gives the desired information as to the vitamin A content of the test material. When rats from our own colony are used, the comparison can be made with about half the number of animals.

SUMMARY.

A type of response to small doses of vitamin A is described. It is a resumption of growth, after the animal has become steady in weight on a deficiency of this factor, which is subnormal in rate, graded to the dose and without premature slackening.

The importance of a long test period, of eight or more weeks, is emphasised.

REFERENCES.

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Steenbock and Coward (1927). *J. Biol. Chem.* **72**, 765.