**CXXII. THE RELATION OF THE GROWTH
RESPONSE TO DOSAGE OF VITAMIN A.
CONFIRMATION OF A CURVE RELATING RESPONSE
TO DOSE OF VITAMIN A GIVEN.**

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COWARD *et al.* [1930] published a curve which related the average increase in weight in 5 weeks of groups of rats which, having ceased to grow on a diet deficient in vitamin A, had then been given graded doses of a certain sample of cod-liver oil. Each group consisted of about 30 rats (nearly equal numbers of males and females) and all the rats of any one group were given the same daily dose of cod-liver oil for 5 weeks. The different groups of rats were given daily doses of 0.25, 1.0, 1.5, 2.5, 7.5 and 20.0 mg. cod-liver oil respectively. The curve through the points obtained by plotting the mean increases in weight of the different groups against the dose of cod-liver oil given was a remarkably smooth one and apparently logarithmic in character. By plotting the mean increases in weight against the logs of the doses given, the points were found to be nearly in a straight line. The best straight line through these points was calculated, $y = 12.1 + 40.2x$; in the form $y = 12.1 + 40.2 \log x$ it applied to the curve itself. Thus it follows that in a comparison of two substances the relative potency of the doses tested is not the ratio of the mean increases in weight of the groups of rats used; it is the ratio of the abscissae corresponding to the mean increases in weight in the curve relating mean increase in weight to dose of vitamin A given; for example if, in a simultaneous test of two samples of cod-liver oil, 1 mg. of one sample given daily for 5 weeks produced a mean increase in weight of 16 g. in a group of 5 male and 5 female rats, and 1 mg. of another sample of cod-liver oil produced a mean increase in weight of 8 g. in a similar group of rats, the ratio of the potencies of the two samples would not be $16:8=2$ (the ratio of the mean increases in weight) but $1.25:0.79=1.58$ the ratio of the abscissae corresponding to the mean increases in weight as obtained from Coward's curve of response.

Use of this curve for comparison of two substances of different kinds.

The curve of response is needed for interpreting the mean increases in weight of two groups of rats which have been given doses of very different substances (e.g. cod-liver oil and, say, milk powder). It is therefore necessary to show that it holds good (*i.e.* measures only vitamin A), no matter what other factors may be unavoidably given to the rats in either or both of the doses of substance under test. That a curve of response may be unreliable and even useless has been shown by Coward *et al.* [1933] in work on the estimation of vitamin B₁. A rather shallow curve of response was obtained when vitamin B₂ *etc.* was

supplied as 8 % yeast autoclaved for 6 hours at 15 lbs. pressure in the basal diet, and the results from pairs of doses of such substances as wheat germ, dried yeast and a certain food product could not be interpreted by it. The result from the higher dose of each substance was much higher than any point on the curve. However, when a fresh curve of response was constructed from results obtained with 20 % autoclaved yeast in the basal diet, a steeper curve of response was obtained than before and the results which could not be interpreted by the first curve could be interpreted by the second. Obviously there had been insufficiency of some factor (B_2 ?) in the first basal diet so that the full effect of the doses of vitamin B_1 alone could not be produced, but the substances tested (wheat germ, dried yeast, *etc.*) had apparently made up the deficiency.

This experience and a similar experience in the construction of curves of response relating increase in weight and dose of vitamin D given [Coward *et al.*, 1932] made us examine all the results of our vitamin A tests in which we had tested two or more doses of any one substance. The results indicate that our curve of response for vitamin A holds good, no matter what may be the nature of the substance tested. This, in turn, indicates that the basal diet used in our vitamin A tests contains enough of all substances which are necessary for growth except vitamin A.

The examination of results from two doses of any one substance.

In each experiment in which we have tested two or more different doses of one substance for vitamin A, we have determined the apparent potencies of the doses tested by finding from the curve of response the abscissae corresponding to the mean increases in weight of the rats used for each dose. These give the apparent ratio of the doses, and by comparison of the apparent ratio with the known ratio some idea of the reliability of the curve of response may be obtained.

In practice we have seldom used the curve of response already described in this paper, for it requires an equal number of male and female rats in each group of animals used. In 1931 we separated the results from the male and female rats in the groups used for constructing the curve of response, and constructed other curves of response for male and female rats separately. The curves were $y = 11.3 + 50.3 \log x$ for the bucks and $y = 12.4 + 27.4 \log x$ for the does. Later, with more information from our tests, these equations became $y = 13.4 + 45.81 \log x$ (bucks) and $y = 12.5 + 27.0 \log x$ (does). Thus we now average separately the increases in weight of the bucks and does given any one dose, find the corresponding abscissae from their respective curves of response and find the weighted mean of these abscissae. This can then be compared with the figure similarly obtained from a group of rats on another dose.

In Table I are collected the results of all experiments in which two or more doses of a substance have been tested for vitamin A. In the last two columns are stated (a) the known ratio of the doses, and (b) the apparent ratio of the doses determined as just described. The average abscissae have been included in the table to show how wide a range of doses the curve may cover.

The results show very good agreement between the known ratio of the doses tested and the apparent ratio of the doses. All the tests but one (dried milk (c)) show better agreement than might be expected from the known error of the test. This is the more satisfactory when the varied nature of the products tested is realised. The products include butter and margarine (very good agreement), two samples of cod-liver oil (less good agreement, one rather high and the other rather low), a fish-meal (very good agreement), four samples of dried milk (two in good agreement, one too high and one too low and therefore not disturbing),

Table I. *Comparison between the known ratio of pairs of doses of different substances tested for vitamin A and the apparent ratio of the doses as determined from the abscissae of the curve of response corresponding to the mean increases in weight found in the tests.*

When the substance was given as a percentage of the diet, records were made daily of the amount of food eaten, and from these were calculated the average amount of test substance eaten per day throughout the experiment.

Substance	Dose	Weighted mean of abscissae for male and female rats	Known ratio of doses	Apparent ratio of doses, i.e. ratio of abscissae
Dairy butter	0.05 g.	1.34	2 : 1	2.5 : 1
	0.1	3.36		
Vitaminised margarine	0.05 g.	1.66	2 : 1	2 : 1
	0.1	3.4		
Cod-liver oil C	1 mg.	0.41	3 : 1	4.3 : 1
	3 mg.	1.77		
Cod-liver oil G	1 mg.	0.55	3 : 1	2 : 1
	3 mg.	1.09		
Fish-meal	2.5 %	2.37	10 : 1	10.8 : 1
	25.0 %	25.6		
(a) Dried milk (0.4 %)	0.022 g.	0.42	7 : 1	12 : 1
	(2.0 %) 0.15	5.05		
(b) „ (0.2 %)	0.013 g.	0.84	12 : 1	8.5 : 1
	(2.0 %) 0.155 g.	7.2		
(c) „	0.1 g.	3.2	9 : 1	3.8 : 1
	0.88 g.	12.0		
(d) „	0.005 g.	0.25	8 : 1	7.5 : 1
	0.04 g.	1.87		
(a) Vitamin prep. 2 %	0.1 g.	0.71	3.6 : 1	2.8 : 1
	5 % 0.36 g.	1.99		
(b) „	0.01 g.	1.3	2 : 1	2.6 : 1
	0.02 g.	3.4		
Wheat embryo 10 % prep.	0.71 g.	0.64	4.5 : 2.2 : 1	6 : 2.7 : 1
	20 % 1.56 g.	1.74		
	40 % 3.2 g.	3.86		

two vitamin preparations (good agreement), a wheat embryo preparation (fairly good agreement). It is perhaps more satisfactory still when it is realised that some of the best results were obtained in tests where the ratios of the doses were greatest, *viz.* 10:1 and 8:1.

We therefore feel some confidence in using these curves of response for comparing mean increases in weight of groups of animals as treated in our vitamin A tests. The basal diet in use in vitamin A tests in this laboratory contains at least enough of each of the factors necessary for growth for the amount of vitamin A in the dose tested to exert its full effect.

The possible use of these curves in other laboratories.

Considering the influence of partial deficiencies of certain factors in the basal diet on the slopes of curves of response, it would be unwise to adopt the curves described in this paper in another laboratory without confirming them. The confirmation would not need as many rats as were used in the construction of these curves. Probably 10 male and 10 female rats on each of 3 doses, fairly widely distributed; for example 0.5, 2.0, 8 mg. of an average sample of cod-liver oil would suffice for this purpose.

DISCUSSION.

We can find only one other report of the influence of the basal diet on the slope of the curve of response to graded doses of a vitamin. Sherman and Munsell [1925] published a series of composite growth curves of groups of rats which had been depleted of reserves of vitamin A and had then been given graded doses of tomato juice. Later Sherman and Batchelder [1931] published a series of composite growth curves of rats similarly prepared and then given graded doses of dried milk powder. They stated that "milk powder experiments showed a more accentuated response than doses of tomato to increasing levels of feeding." It is a simple matter to construct curves of response for 1, 2, 3, 4, 5, 6, 7 or 8 weeks' feeding from the figures of their two papers. We constructed the curves of response for 5 weeks' feeding, partly for comparison with our own curve, and partly because Sherman and Batchelder suspected that the rats of the most rapidly growing group suffered from a shortage of the "Coward factor" in the last week or two of the test. We could construct only one curve of response for each test, and not two separate ones for male and female rats respectively as the details for male and female rats were not given separately. Each group, however, consisted of approximately equal numbers of bucks and does. The curve of response to graded doses of dried milk powder was very much steeper than that to graded doses of tomato juice, and Sherman and Batchelder say "the less accentuated response to increasing levels of feeding of the tomato may have been because this was a less adequate source than milk of some factor or factors whose influence upon growth was not fully realised at the time of the earlier experiments, or possibly that, notwithstanding the general equivalence of the vitamin A of animal origin, and its precursor of vegetable origin, there may still be a difference in the degree of readiness and completeness of utilisation of the two forms." It is of interest to note that the basal diet used in Sherman's tomato experiment contained only 5 % of dried brewer's yeast, whereas the diet used in the milk experiment contained 10 % dried brewer's yeast. The greater "spread" of the results of the tests of graded doses of dried milk may have been due to the larger supply of some factor in the dried yeast or to some factor other than vitamin A supplied by the milk and not contained in the basal diet. Whichever was the cause, the results demonstrate afresh the necessity of using a basal diet which has been proved to contain sufficient of all factors necessary for growth except the one for which a test is to be made.

Morgan has constructed curves of response relating mean increase in weight in 5 weeks of rats which, having nearly ceased to grow on a diet similar to our vitamin A-free diet, have then been given graded doses of vitamin A (private communication). They are $y_1 = c_1 + 37.6 \log x$ (bucks) and $y_2 = c_2 + 26.1 \log x$ (does), but as his rats fed on this diet do not cease to grow on the basal diet alone, the slopes of the curves cannot fairly be compared with our own.

The following equations of the curves of response of similar tests for vitamin A, so far available for comparison, are therefore:

- (a) Sherman's tomato juice curve, $y = 9.9 + 22.6 \log x$.
- (b) Sherman's dried milk curve, $y = 5.3 + 69.8 \log x$.
- (c) Coward's curve, $y = 12.1 + 40.2 \log x$.

It appears to be easy to explain the difference in slope of Sherman's two curves on the grounds of insufficiency of some factor in the basal diet, but it is difficult to see how this could be the reason for Coward's curve being less steep than Sherman's steeper one, for it has been shown that Coward's curve holds

good for graded doses of substances containing many different growth factors in addition to vitamin A. Does Sherman's very steep curve of response to graded doses of dried milk powder indicate that when rats have been fed on a diet partially deficient in one factor and wholly in another factor for several weeks and are then given abundance of the former and graded doses of the latter, the response will be greater than if the rats had had abundance of the first factor throughout the experiment? It is impossible to say if this is so from the experiments so far recorded. It can only be emphasised again that if a curve of response is to be used for determining relative potencies of doses, it is of the utmost importance that the basal diet should contain abundance of all factors necessary for growth except the one for which the test is being made.

SUMMARY.

The validity of Coward's curve of response relating mean increase in weight in 5 weeks to dose of vitamin A given has been confirmed by the method of comparing the known ratio of two doses of a substance tested with their apparent ratio as obtained from the abscissae of the curve of response corresponding to the mean increases in weight of the rats given the respective doses.

Twelve substances have been tested in this way. They included butter, vitaminised margarine, fish-meal, cod-liver oil, dried milk, wheat embryo, *etc.*

The agreement between known ratio of doses and apparent ratio of doses left no doubt that the curve was reliable, especially in view of the varied nature of the substances tested and the very large ratio of some of the pairs of doses used (*e.g.* 10:1, 8:1).

The range of doses which can be compared by the use of this curve extends from a dose which is just sufficient to maintain weight of rats which have ceased to grow on a diet deficient in vitamin A, to a dose which is 50 times as great. There is no "flattening" of the curve, and it is therefore concluded that there is no deficiency in the basal diet except vitamin A.

Comparison with curves of response from other laboratories has been made and differences in slopes of curves of response have been discussed.

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