CCLXXXII. THE ACCURACY OF VITAMIN A DETERMINATIONS. THE STARTING-POINT OF THE TEST PERIOD.

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(Received 30 September 1936.)

Most vitamin A tests in this country are made by the "increase in weight" method; i.e. young rats which have become steady in weight on a diet deficient in vitamin A are given doses of the test substance or standard for a specified period. The resultant increases in weight are used to determine the potency of the test substance in terms of the standard of reference.

Some discussion has arisen in the last few years as to the best point of each animal's weight curve to consider as the beginning of the "increase in weight". Coward and co-workers have always used the point of the curve at which the animal was considered steady in weight and given the first dose of vitamin A. The test period, i.e. the period during which doses of vitamin A were given, was then always of the same length (5 weeks in early work, reduced later to 3 weeks). Baumann *et al.* [1934] obtained good results by counting the point at which the rat's weight passed the highest point reached in the preparatory period as the beginning of the test period and ignoring the part of the rat's curve where it was regaining this weight. Eddy [1936], however, decided that the method of calculating the 28 days' gain had little influence on the accuracy of the test. Various other workers have suggested (verbal communications) that this method of counting the period of the test might give more accurate results than counting it from the first day of dosing. In this method the time during which doses were given to some of the rats would have to be prolonged beyond the set period of the test. Some rats might never (within the limits of the patience of the worker) reach the needed starting-point for the test and they would have to be discarded.

It seems possible to test this suggestion for increasing the accuracy of a determination by the following consideration. The recovery, if any, to the suggested starting-point would generally take place within the first 2 weeks of dosing. Therefore, the accuracy obtainable in the last 3 weeks (say) of a 5 weeks' test, should be greater than that obtainable in the first 3 weeks of the test which included all the responses of the type described. A calculation has been made of the accuracy of the test obtainable during each of the 5 weeks of dosing. This was based on the calculation of $\lambda = \sigma / \text{the slope of the line relating increase in weight to } \log_{10} \text{ of dose given}$. Coward [1933] showed that the relation between the mean variance of the increase in weight of rats and the duration of the test period was a straight line for the whole of the 5 weeks for male rats, and also for the first 3 weeks for female rats, though the curve for these flattened a little in the fourth and fifth weeks. Thus the variance increased by an equal amount during each week of the test period. Therefore the variance in the increase in weight during the first, second, third, fourth and fifth weeks was the same for the male rats, and for the female rats slightly less in the fourth and fifth weeks than in the first 3 weeks. Hence the standard deviation of the increase in weight during the first, second, third, fourth and fifth weeks was the same for the male rats, and for the female rats slightly less in the fourth and fifth weeks.

The curves for the first week have already been published [Coward, 1933]. It remained then to determine the curves of response to doses of vitamin A during the second, third, fourth and fifth weeks, both for the male and female rats. This was done by the use of the formula

$$\tan (\alpha - \beta) = \frac{\tan \alpha - \tan \beta}{1 + \tan \alpha \tan \beta},$$

where, for example,

$\tan \alpha$ was the slope (b_1) of the curve of response for a 2 weeks' test;

$\tan \beta$ was the slope (b_2) of the curve of response for a 1 week's test;

$\tan (\alpha - \beta)$ was the slope (b) of the curve of response for the increase in weight during the second week.

The values of b (the slopes of the curves) in the equation had to be divided by 100 to reduce the unitage of y to that of the abscissae. The slopes of the curves of response to doses in the first week, and in the second, third, fourth and fifth weeks found by this method may be seen in Table I.

Table I. *The slopes of the curves of response relating increase in weight during the first, second, third, fourth and fifth weeks respectively to the log of the dose of vitamin A given.*

	Male rats	Female rats
1st week	9.05	6.64
2nd week	10.05	6.65
3rd week	9.78	4.37
4th week	7.59	4.37
5th week	6.59	4.26

It is evident that with the exception of the first week's response of the male rats and perhaps the third week's response of the female rats, the two sets of curves fall into two nicely graded series. The inaccuracy of the test may be measured by

$$\lambda = \frac{\sigma}{\text{slope of curve of response}},$$

and since σ is constant, then the greater the slope of the curve of response, the less is the inaccuracy of the test. The smaller value of σ in the fourth and fifth week's variance of the response of the does lowers the values for λ very little. Therefore the conclusion must be drawn that each successive week's dosing gives a slightly less accurate result than that of the week preceding it. It would, therefore, seem to be preferable to treat the responses to doses of vitamin A in the simplest way, i.e. to count the period of the test from the day on which the first dose is given. This prevents the prolongation of a test for the sake of a few rats, which is an economy of time and which also prevents the possibility of the results from these same rats being influenced by changes in conditions which would not affect the results from periods already completed.

This calculation was based on figures obtained from 960 male rats and 1110 female rats.

SUMMARY.

Different workers have suggested that the recovery period in a vitamin A determination should be taken as starting on that day on which the rat receiving doses of vitamin A exceeds the highest weight it had attained during the preparatory period when it was not receiving any vitamin A.

This suggestion has been tested on a colony of rats in which all the animals lost weight (some of them only a few g.) before they were given doses of vitamin A, and many of them continued to lose weight for a short time before

recovery began. With most animals the point suggested above as the starting-point for the calculation of increase in weight was attained within 1 or 2 weeks (if ever) from the day on which vitamin A was first given. Hence a calculation of the accuracy of the results obtained during successive weeks should indicate whether it is desirable or not to ignore the period previous to the point suggested as the starting-point for the calculation.

The accuracy obtainable during each week of a 5 weeks' test period is determined from $\lambda = \sigma / \text{slope of curve of response}$. It has been shown that since σ is the same for successive weeks and the slope of the curve of response for each week's increase in weight becomes slightly less in successive weeks, the accuracy of the result of each week's dosing is slightly less than that of the preceding week. Hence the greatest accuracy is obtained by counting the test period from the first day of dosing.

REFERENCES,

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