CCLXXXIII. THE INFLUENCE OF THE LENGTH OF THE TEST PERIOD ON THE ACCURACY OBTAINABLE IN A VITAMIN B₁ TEST.

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It was shown by Coward [1933] that the accuracy obtainable in a vitamin A test by the "increase in weight" method was increased very little by prolonging the test period beyond three weeks. Since then, enough results of vitamin B₁ determinations by the method of the "increase in weight" of rats have been accumulated to make it possible to carry out a similar examination of the duration of a vitamin B₁ test.

The technique of the test has been described by Coward *et al.* [1933]. Briefly, it consists of measuring the increase in weight of rats which, having become steady in weight on a diet deficient in vitamin B₁, are then given doses of (a) the International Standard (I.S.) for vitamin B₁ or (b) the substance under examination. With the description of the test was published an estimate of the accuracy obtainable. It was much greater than that obtainable in a vitamin A test of the same length of time. Thus a smaller number of animals would be required for a vitamin B₁ test for a given degree of accuracy than would be required for a vitamin A test of similar accuracy.

In the determination of the vitamin B₁ content of a substance we have generally divided the rats into 4 or 5 groups, two of the groups being given doses of 0.01 and 0.02 g. respectively of the I.S. and two of the groups doses in the ratio of 1 : 2 of the substance under examination or, if no information on the possible potency of the substance was available, the doses chosen for a preliminary test were in the ratio of 1 : 3 or 1 : 5. A group of rats given no dose was included when it was desired to show whether even a large dose of the test substance was or was not better than no dose at all. The different groups have generally contained equal numbers of bucks and does but it was shown in the paper by Coward *et al.* [1933] that the curve of response of bucks was very little steeper than that of does; hence it was not really necessary to have equal numbers of each in each group. By this means we have obtained a large amount of information not only on the variation in response to doses of vitamin B₁ but also on the variation in the slopes of the curve of response. It has confirmed our previous estimate of the high degree of accuracy attainable and it has shown that the accuracy obtainable by carrying on the test for three weeks is only very slightly greater than that obtainable by carrying it on for two weeks.

The average variance (σ^2) of the increase in weight in 1, 2 and 3 weeks.

The average variance of the response of male and female rats to doses of vitamin B₁ was calculated by the formula usually employed:

$$\sigma^2 = \frac{\Sigma d^2}{N - M},$$

(2012)

where Σd^2 is the sum of the squares of all the deviations from their respective means; N is the number of rats from which the calculation was made; M is the number of groups of rats in which the N rats were distributed.

The results for male and female rats for 1, 2 and 3 weeks' tests are collected in Table I.

Table I. *The average variance and standard deviation of male and female rats to doses of vitamin B₁ in 1, 2 and 3 weeks' tests.*

		Σd^2	N	M	σ^2	σ
Male rats	1 week's test	4132.0	306	110	21.08	4.31
	2 weeks' test	8512.5	295	105	44.80	6.69
	3 ,,	12050.5	282	101	66.58	8.16
Female rats	1 week's test	3660.5	243	96	24.90	4.99
	2 weeks' test	5628.0	229	92	41.08	6.41
	3 ,,	7173.0	215	85	55.18	7.43

The average variance of response in 1, 2 and 3 weeks when plotted against the time gives a nearly straight line relationship for both the male and female rats. The line for the male rats has a greater slope than that for the female rats, but the two lines intersect. That for the female rats does not pass through or near the origin. (Compare with the slopes of the lines relating average variance in response to doses of vitamin A with the duration of the test [Coward, 1933].)

The standard error of the average result from 10 animals is $\frac{\sigma}{\sqrt{10}}$, the probable error is $\frac{2}{3} \cdot \frac{\sigma}{\sqrt{10}}$ and the probable error of the difference between the average responses of two groups of rats (e.g. one given a dose of the standard and one given a dose of test substance) is $\frac{2}{3} \cdot \frac{\sigma}{\sqrt{10}} \cdot \sqrt{2}$.

The curves of response of male and female rats to doses of vitamin B₁ in 1, 2 and 3 weeks.

The difference in the average responses to all the pairs of doses in the ratio 1 : 2 of all substances tested were calculated for the 1, 2 and 3 weeks' tests. (The response of any animal that died during the 3 weeks was used for the 1 or 2 weeks previous to death. No result from it was counted in the week of its death.) The weighted mean of these differences was determined. This was permissible since it had been shown by Coward *et al.* [1932] that the line relating increase in weight in 3 weeks to dose of vitamin B₁ given is logarithmic. This average value is the difference in increase in weight due to a doubling of the dose of vitamin B₁ given. Therefore, in the curve of response relating increase in weight to the logarithm

Table II. *The calculation of the slopes of the curves relating increase in weight in 1, 2 and 3 weeks to the logarithm (to the base 10) of the dose of vitamin B₁ given.*

		Average difference between increases in weight from doses in ratio 1 : 2	Slope of curve of response (Diff./log 2)
Male rats	1 week's curve	6.23	20.70
	2 weeks' curve	11.09	36.88
	3 ,,	15.88	52.76
Female rats	1 week's curve	5.28	17.54
	2 weeks' curve	11.94	39.67
	3 ,,	16.86	56.01

of the dose of vitamin B₁ given, the interval for the average value of y corresponds to an interval of 0.30103 ($\log_{10} 2$) for x . The slopes of the curves of response are then obtained by dividing the interval (average differences in the increase) by 0.30103 (Table II).

There is not as great a difference between the slopes of the curves relating increase in weight of male and female rats to doses of vitamin B₁ as there is to doses of vitamin A or Vitamin D [Coward, 1933; Coward *et al.*, 1932].

The determination of the accuracy obtainable in 1, 2 and 3 weeks' tests.

The accuracy obtainable by carrying on the test for 1, 2 or 3 weeks is determined from the formula $\lambda = \sigma / \text{slope of curve of response}$ (Table III). The probable error of a determination in which 20 animals were used, ten of them being given a dose of the standard and the other ten a dose of test substance is determined from the formula

$$\frac{2}{3} \cdot \frac{\sigma}{\sqrt{10}} \cdot \frac{\sqrt{2}}{\text{slope}} \quad \text{or} \quad \frac{2}{3} \cdot \frac{\lambda}{\sqrt{10}} \cdot \sqrt{2}.$$

Table III. *The accuracy obtainable by carrying on the test for 1, 2 or 3 weeks with 10 pairs of animals, one of each pair having been given a dose of the standard and the other a dose of test substance.*

		σ	Slope of curve	$\lambda = \sigma / \text{slope}$	$\frac{2}{3} \cdot \frac{\lambda}{\sqrt{10}} \cdot \sqrt{2}$	Antilog range of dose ratio	Range of probable error of determination %
Male rats	1 week's test	4.31	20.70	0.208	0.0620	1.153	+15.3
					1.9380	0.867	-13.3
	2 weeks' test	6.69	36.88	0.181	0.0539	1.132	+13.2
					1.9461	0.883	-11.7
	3 „	8.16	52.76	0.155	0.0462	1.112	+11.2
					1.9538	0.899	-10.1
Female rats	1 week's test	4.99	17.54	0.284	0.0846	1.215	+21.5
					1.9154	0.823	-17.7
	2 weeks' test	6.41	39.67	0.162	0.0483	1.118	+11.8
					1.9517	0.895	-10.5
	3 „	7.43	56.01	0.133	0.0396	1.096	+ 9.6
					1.9604	0.913	- 8.7

Thus the probable error of a determination of vitamin B₁ in which ten rats are used for the standard and ten for the substance whose potency is being determined is less in a 2 weeks' test than in a 1 week's test, and only slightly less in a 3 weeks' test than in a 2 weeks' test. Indeed the increase in accuracy gained by carrying on a test for a third week cannot be considered worth the extra labour involved. With equal numbers of male and female rats on standard and test substance, the ratio of the accuracies of 1, 2 and 3 weeks' tests is about 18 : 12.5 : 10 %.

The number of animals on which this calculation was based was 549 for the 1 week's test, 524 for the 2 weeks' test and 497 for the 3 weeks' test, some of the rats on the low doses having died during the course of the experiments.

SUMMARY.

The average variance in response of rats to doses of vitamin B₁ has been determined for tests of 1, 2 and 3 weeks' duration.

The slopes of curves of response relating increase in weight in 1, 2 and 3 weeks and dose of vitamin B₁ given have been constructed by averaging the differences in response obtained by giving two doses in the ratio 1 : 2 of numerous substances. This method is justified by its having been shown in a previous paper that the relation between increase in weight and dose of vitamin B₁ given is logarithmic.

The accuracy obtainable in the test when carried on for 1, 2 and 3 weeks has been determined by the formula $\lambda = \sigma / \text{slope of the curve of response}$. The probable errors of determinations of the vitamin B₁ potency of a substance by comparison with the standard tested simultaneously, when 10 animals are used on each substance, and when the tests are carried on for 1, 2 and 3 weeks, are about 18, 12.5 and 10% respectively. Thus the increased accuracy obtained by carrying on a test for longer than 2 weeks is seldom worth the extra labour involved.

REFERENCES.

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