CXXIII. SIMPLIFICATION OF VITAMIN TESTS. THE EFFECT OF GIVING DOSES HALF-WEEKLY FOR VITAMIN A TESTS, AND ONCE ONLY FOR VITAMIN D TESTS, INSTEAD OF GIVING DOSES DAILY.

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(Received April 30th, 1934.)

THE most laborious part of the work involved in testing for vitamins is, perhaps, the giving of a daily dose of the standard or of the substance under test to the experimental animals. It occurred to us that a great saving of labour would be effected if the doses could be given at less frequent intervals. We therefore carried out two experiments:

1. On the effect of giving (a) daily doses, and (b) half-weekly doses in a 5 weeks' test for vitamin A (resumption of growth).

2. On the effect of giving (a) daily doses, and (b) the whole of the 10 days' dose on the first day of the 10 days' curative period in a vitamin D test.

Both experiments indicated that the giving of doses every day in tests for vitamins A and D is unnecessary; the doses can safely be given at longer intervals than 24 hours, though we have not attempted to determine the limits of these intervals.

Moreover, since adopting the simpler method of dosing, we have obtained enough figures to show that it is at least as accurate as the more laborious method of dosing every day.

We have not extended our investigation to the water-soluble vitamins. It is quite probable that, owing to the nature of their action in the animal body (so far as it is at present understood), the giving of doses at longer intervals than 24 hours would not have the same effect as giving the doses daily.

I. VITAMIN A TEST.

A. Comparison of effects of giving doses (a) daily and (b) half-weekly.

The technique employed in this test was that described by Coward *et al.* [1930]. A cod-liver oil, chosen for examination by the two methods of dosing, was diluted with olive oil so that one drop of the dilution (19–21mg.) contained 1 mg. cod-liver oil. Some rats which had ceased to grow on a vitamin A-deficient diet were given one drop of this solution daily, others were given 3 drops on Mondays and 4 drops on Thursdays. The mean increases in weight of the bucks and does were calculated separately for each group (Table I).

Table I. *Mean increase in weight of groups of rats which had become steady in weight and were then given cod-liver oil (a) daily, or (b) half-weekly, the total dose for each rat being 7 mg. per week for 5 weeks.*

Method of dosage	No. of rats		Mean increase in weight in 5 weeks	
	♂	♀	♂	♀
Daily	5	6	19.6	14.3
Half-weekly	4	7	20.0	14.0

It is obvious that the method of dosing half-weekly has given the same result as the method of dosing daily.

B. *Validity of this result.*

The possibility that two results in our vitamin A tests although apparently equal, may in fact be different, has been worked out for groups of 10 animals. The larger the number of animals in each group, the greater is the probability that the results are indeed alike.

First were calculated the probabilities that any result with a group of rats would be greater or less than the true result by given amounts; *e.g.* Table II shows the calculation by which it was found that there was a 1/22 chance that 10 male rats would give a result either greater than 153.7 % or less than 65.1 % of the true value and that 10 female rats would give a result either greater than 179.8 % or less than 55.6 % of the true value.

Table II. *Calculation of the probability (1/22 chance) that the result of a vitamin A test (a) based on a group of 10 male rats would be greater than 153.7 or less than 65.1 % of the value found, and (b) based on a group of 10 female rats, would be greater than 179.8 or less than 55.6 % of the value found.*

	Bucks	Does
Standard deviation of the increase in 5 weeks of the rats of this colony in vitamin A tests	14.72	11.02
Curve of response relating mean increase in weight in 5 weeks to dose of vitamin A	$y = 11.3 + 50.3 \log x$	$y = 12.4 + 27.4 \log x$
Logarithmic variance of the test (Standard deviation/slope of curve of response)	$\lambda_1 = \frac{14.72}{50.3} = 0.293$	$\lambda_2 = \frac{11.02}{27.4} = 0.402$
Log. var. of mean when 10 rats are used in a test	$\frac{\lambda_1}{\sqrt{10}} = 0.093$	$\frac{\lambda_2}{\sqrt{10}} = 0.127$
Probability (1/22 chance) that a result with 10 rats would be:	2.007×0.093 $= 0.1867$ $= \log 1.5371$ greater than 153.7 % or less than 65.1 % of the true value	2.007×0.127 $= 0.2549$ $= \log 1.7984$ greater than 179.8 % or less than 55.6 % of the true value

* Table of x , Fisher, *Statistical Methods for Research Workers*, p. 76.

In a test made with 5 bucks and 5 does, there is a 1/22 chance that the true value is either less than 60.3 % (average of 65.1 and 55.6) or greater than 166.7 (average of 153.7 and 179.8) of the value found. There is a 1/44 chance that it would be one or the other. In a comparison of 2 doses with 2 groups of rats (5 bucks and 5 does in each), there is a $1/44 \times 1/44$ chance that the true value of one dose is 60.3 % of the found value while the true value of the

other dose is 166.7 % of the found value, *i.e.* there is a 1/1936 chance that one value is really more than $166.7/60.3 = 2.76$ times the other. This probability is very small indeed. Other probabilities are shown in Table III.

Table III. *Chances that the true ratio of two doses apparently equal may in fact be greater than 1:1.*

Probability	True ratio	Probability	True ratio
1 in 1936	2.76	1 in 144	2.02
1 in 900	2.57	1 in 36	1.63
1 in 400	2.30	1 in 16	1.41
1 in 256	2.18	1 in 4.4	1.03
1 in 196	2.10		

Thus there is only a 1 in 4.4 chance that either of two results which are apparently equal may be more than 3 % greater than the other. This is much smaller than the "probable error" (1 in 2 chance) which is the probability most often used for describing the accuracy of a biological test. There is a 1 in 16 chance of one result being more than 40 % greater than the other. This again is a small chance. We, therefore, felt justified in changing from daily doses to half-weekly doses for vitamin A tests.

The calculation just described was based on the assumption that the standard deviation of the increase in weight in 5 weeks of rats given half-weekly doses of vitamin A was the same as that of rats given daily doses of vitamin A. We have not enough figures to calculate the standard deviation of the increase in weight of rats given doses half-weekly for 5 weeks, but since adopting this method of dosing we have gained a certain amount of information from which we have calculated the standard deviation of the increase in weight of rats given doses half-weekly for 3 weeks. This figure can be compared with the standard deviation of the increase in weight of rats given doses daily for 3 weeks (Table IV).

Table IV. *Standard deviation of the increase in weight in 3 weeks of rats which have been given doses of vitamin A (a) half-weekly, (b) daily.*

Doses given		No. of tests	No. of rats	Σd^2	Average variance	Standard deviation
Half-weekly	Bucks	78	326	41,936	169.8	13.03
	Does	83	430	30,958	89.22	9.45
Daily	Bucks	184	1014	118,764	143.09	11.96
	Does	194	1187	95,616	96.29	9.81

Thus the standard deviation of the increase in weight in 3 weeks was slightly higher for the bucks and slightly lower for the does which were given doses of vitamin A half-weekly, than for the corresponding rats which were given doses daily. It was, therefore, reasonable to use the standard deviation of the increase in weight of rats given daily doses for 5 weeks for calculations relating to the increase in weight of rats given half-weekly doses for 5 weeks.

II. VITAMIN D TEST.

A. *Comparison of effects of giving (a) one dose at the beginning of the 10 days' curative period, and (b) 1/10 of that dose daily for 10 days.*

We made a direct comparison of these two methods of dosing first of all by using the standard solution of vitamin D. This showed that the two methods gave the same result, but we made another comparison of the two methods

using cod-liver oil (our oil Z) lest the finding with the standard should not be applicable to cod-liver oil. This experiment also gave the same result by the two methods and we, therefore, felt justified in adopting the simpler method of dosing in all estimations of the vitamin D content of samples of cod-liver oil.

(a) *Standard solution of vitamin D.* 19 rats from 8 different litters (approximately half of each litter) which had received a rachitogenic diet (Steenbock's 2965) for $3\frac{1}{2}$ weeks were each given 5 units of the International Standard solution of vitamin D in one drop (20 mg.) of solution. They were given the basal diet without addition for the following 10 days. 20 rats, the other halves of the same litters, having as nearly as possible the same initial weights (*i.e.* paired), were given 0.5 unit standard vitamin D in 20 mg. solution daily. Ten days after dosing began, all the animals were killed and their ulnae and radii were examined by the method in general use in this laboratory [Dyer, 1931; Key and Morgan, 1932]. The average degree of healing of the 19 rats which received 5 units of vitamin D in one dose was 3.00 and the average degree of healing of the 20 rats which received 0.5 unit daily was 3.20. Thus the two methods gave almost the same result.

(b) *Cod-liver oil.* 10 rats from 3 litters (half of each litter) were each given 20 mg. cod-liver oil in one dose on the first day of the curative period, and 10 rats, the other halves of the same litters, were each given 2 mg. of the same cod-liver oil for 10 days. The average degree of healing, determined as before, was found to be 2.0 for the animals receiving one 20 mg. dose and 1.7 for those receiving 2 mg. daily. Thus the two methods of dosing again gave almost the same result (Table V). Moreover, the method, which gave a slightly higher result in one experiment, gave a slightly lower result in the other. It, therefore, seemed justifiable to conclude that the two methods gave equal results.

(c) *Other substances.* Most substances other than liver oils and vitamin D concentrates contain so little vitamin D that large doses of the substance have to be given in order to produce an effect. Thus butters containing from about 0.5 to 2.0 units per g. have to be tested in doses of 0.5 g. per day. This is about as much butter as can be conveniently given to a rat at one time; hence we have continued to give doses of butters and of most other substances every day as before.

Table V. *Comparison of the effects of giving (a) the whole dose at the beginning of the 10 days' curative period, and (b) a daily dose for 10 days' curative period.*

	Average healing in groups of rats after	
	(a) Whole dose at beginning of 10 days' curative period	(b) Daily dose for 10 days' curative period
Irradiated ergosterol (5 units altogether)	3.0	3.2
Cod-liver oil (20 mg. altogether)	2.0	1.7

B. *Comparison of the two methods of dosing in vitamin D tests with regard to the accuracy obtainable in the result.*

When we began to give the whole of the 10 days' dose of oil or standard at the beginning of the curative period, we delivered the dose from a micrometer syringe, wiping the nozzle carefully before putting it sideways into the rat's mouth and then wiping it once or twice on the side of the rat's mouth on withdrawal, to make sure that the rat had received the whole of the very small dose.

The volumes of the doses to be delivered were calculated from the average specific gravity of cod-liver oil and of the diluted standard as determined in this laboratory.

When we had used this method for some months we gained the impression that we were obtaining more uniform results than we had previously. A calculation of the standard deviation of the amount of healing, based on observations of 334 rats, gives a standard deviation 0.716. The standard deviation obtained for tests in which doses were given daily was 0.783. The improvement in accuracy is thus very slight; it reduces the probable error of the result, when 20 rats are used in an estimation, from 9 to 8 %. We are, therefore, satisfied that the method of giving the whole dose at the beginning of the 10 days' curative period gives a result which is not less accurate than that obtained by the method of giving doses daily.

We have applied this result to the routine of our vitamin A tests also. Whereas we used to give each rat which was being used for a vitamin A test a dose of 4 units vitamin D in 20 mg. olive oil twice a week, nowadays we give 8 units of vitamin D in 20 mg. olive oil once a week. This is a saving of labour also.

SUMMARY.

The same results were obtained in a vitamin A test on a certain sample of cod-liver oil by giving the required amount either as daily doses or as half-weekly doses.

A statistical calculation showed that the probability that these results are really equal and not only apparently so is very high. There is approximately a 77 % chance that the results are within 3 % of each other. There is a 94 % chance that they are within 41 % of each other.

A further calculation of the standard deviation of the increase in weight during the test, since this method of dosing was adopted in this laboratory, shows that the method gives results of approximately the same degree of accuracy as did the former method. The standard deviation of the results from the bucks was slightly greater, that from the does slightly less, than the corresponding figures obtained by the other method of dosing.

Practically equal results were also obtained in two vitamin D experiments ((a) using a certain sample of cod-liver oil, and (b) using the International Standard for vitamin D) when the whole dose was given at the beginning of the 10 days' curative period and when the amount was divided into 10 daily doses. The former of these methods of dosing appeared by calculation to be slightly more accurate than the latter.

A great saving of labour in tests for vitamins A and D can be safely effected by giving doses at less frequent intervals than the usual one of 24 hours. There is no evidence yet that this result is applicable to tests on the water-soluble vitamins.

REFERENCES.

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