XLII. SIMPLIFICATION OF VITAMIN TESTS. HALF-WEEKLY INSTEAD OF DAILY DOSES IN VITAMIN B₁ TESTS ON RATS

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COWARD & KEY [1934] showed that in vitamin A tests the same result was obtained by giving doses of vitamin A-containing substances half-weekly as by giving doses daily. They also showed that in a 10 days' curative test for vitamin D, the whole of the 10 days' dose given at the beginning of the curative period produced the same result as if it had been divided into 10 daily doses. On the other hand, Bacharach *et al.* [1936] found a slightly greater effect by giving vitamin D doses daily than by giving the whole dose at once. It is probably immaterial which method is adopted provided that both test substance and standard are treated in the same way. If the test substance is so potent that the whole of the dose can be given on 1 day in a vitamin D test or half-weekly in a vitamin A test, much time and labour can thus be saved.

As the number of routine tests for vitamin B₁ increased in this laboratory, it seemed worth while to compare the two methods with respect to vitamin B₁. A preliminary comparison between the effects of daily doses and half-weekly doses of dried yeast was first made, and then three further comparisons were made using (a) a small dose (0.01 g.), (b) a larger dose (0.02 g.) of an adsorption product of vitamin B₁ and (c) a dose of 5γ crystalline vitamin B₁. In all 4 experiments it was found that the same effect, or nearly the same, was produced by daily doses as by half-weekly doses, the equivalent of 3 daily doses being given 1 day and of 4 being given 3 days later. Where a difference was found it was insignificant. Moreover, it was shown in the second and third experiments, by measuring the amount of food eaten daily, that the giving of vitamin B₁ half-weekly was not accompanied by a lower intake of food on the days when it was not given and a higher intake on the days when it was given, i.e. the effect of vitamin B₁ on the appetite was not confined to the day of administration.

EXPERIMENTAL

Preliminary experiment with the vitamin B complex

Eight rats were prepared for this experiment by giving them a vitamin B (complex)-free diet until they ceased to grow. The diet consisted of:

Physiological caseinate (Glaxo)	...	15 %
Dextrinized rice starch		79 %
Agar-agar		2 %
Salt mixture (Steenbock's 40)	...	4 %

In addition each rat was given 6 drops of a good sample of cod liver oil twice a week. The rats weighed about 60 g. at the beginning of the depletion period and about 70 g. when they ceased to grow. They were kept 4 in a cage during this time, but in separate cages when doses were given. All cages were provided

with grids of about $\frac{1}{2}$ in. mesh, standing 1 in. from the floor of the cage. During the test period the rats were weighed daily. No records were kept of amounts of food eaten. The daily dose of dried yeast was 0.2 g., the half-weekly doses were 0.6 g. and, 3 days later, 0.8 g. All the rats were ready for test on the same day.

One of the rats given daily doses died after 10 days. The other three increased in weight by an average of 21.3 g. in 3 weeks and 23.7 g. in 25 days. The 4 rats given half-weekly doses made an average increase in weight of 24.2 g. in 3 weeks and 21.5 g. in 25 days. This was considered a good indication that the effect of giving doses of the vitamin B complex half-weekly was equal to the effect of giving doses daily. It was decided to repeat the experiment with vitamin B₁.

Fig. 1. Composite curves showing the increase in weight of groups of rats which had become steady in weight on a diet deficient in the vitamin B complex and had then been given doses of dried yeast (a) daily, or (b) the same total amount in half-weekly doses.

Another point of interest was made evident when the curves of increase in weight of the two groups were plotted on the same graph (see Fig. 1). The rats that were given doses of dried yeast half-weekly increased most in weight in the 24 hr. immediately following each dosing, but the rats which were given doses daily increased most in weight on the same days as the rats receiving doses half-weekly. Thus it was not the giving of the high, half-weekly doses that brought about the sudden increases in weight. The cause of the increases was not explained.

A similar parallelism in the increases in weight was evident in the next two experiments, although the greatest increases in weight did not always occur in the 24 hr. following the giving of the half-weekly doses.

First experiment with the vitamin B₁ adsorbate

Twenty rats were prepared as in the last experiment, except that 25 g. of dried brewer's yeast autoclaved in layers about $\frac{1}{4}$ in. deep at 120° for 6 hr. were added to 100 g. of the vitamin B-free diet. In this experiment records were

made of the amount of food eaten by each rat per day during the period when doses of the adsorbate were given. Twice as much food was given on Saturdays to save work on Sundays.

All these rats were ready for test on the same day. Thus it was possible to make certain observations on the average weights and food intake that would have been obscured if the tests had begun on different days; these may be seen in Fig. 2.

The vitamin B₁ powder was an adsorbate containing 200 i.u. vitamin B₁ per g. The daily dose given to each of 10 rats was 0.005 g. To facilitate dosing the powder was diluted 1 in 10 with finely powdered dextrinized rice starch. To each dose weighed out on to a small dish was added about 1 ml. water to prevent it from being blown away. The half-weekly doses were 0.015 and 0.02 g. of the adsorbate respectively, measured from the same diluted sample. The average increases in weight of the rats (male and female) are summarized in Table I.

Table I

	Av. increase in wt. in 3 weeks (g.)	
	♂	♀
Daily dose	- 11.25 (4 rats)	- 11.1 (6 rats)
Half-weekly dose	- 6.6 (5 rats)	- 7.8 (5 rats)

Since the difference between the results from the male rats was 1.68 times the square root of the sum of the squares of the standard errors of the two results, it was not significant; since for the female rats this ratio (*t*) was 2.37, the difference might be just significant.

Fig. 2. Composite curves of weight and daily intake of food of rats which had become steady in weight on a diet deficient in vitamin B₁ only and were then given doses of a vitamin B₁ adsorbate, (a) daily, or (b) the same total amount in half-weekly doses. Dose of vitamin B₁ sufficient to maintain life but not sufficient to produce increase in weight.

In Fig. 2 the composite curves of growth of the rats (♂ and ♀ separately) may be seen, together with the average amounts of food eaten daily. The days on which the half-doses were given are indicated by arrows. From the chart may be seen (a) that the general slope of all 4 curves is about the same, (b) that

increases in weight accompany increases in food consumed, (c) that there is no sudden rise in the average weight of the animals or in the average amount of food consumed immediately after giving a large (triple or quadruple) dose of vitamin B₁, (d) that the fluctuations in average weights of the 4 groups were approximately parallel.

It was satisfactory to find that there was no real difference between the results of giving doses daily and half-weekly. It was, perhaps, to be expected that increases in weight would follow increases in food consumed. It was certainly unexpected to find no immediate evidence of increase in appetite after giving large doses of vitamin B₁. It was decided to repeat this experiment, giving larger doses of vitamin B₁, i.e. large enough to bring about an increase in weight, not merely large enough to maintain weight.

Second experiment with the vitamin B₁ adsorbate

The procedure in this experiment was the same as in the last, except that records of food eaten were kept during the preparatory period (when the rats were receiving no vitamin B₁), as well as during the test period, and doses were given and rats were weighed on Sundays as well as on other days. The rats were kept in separate cages and weighed daily throughout the whole time. The daily dose of vitamin B₁ was 0.01 g. vitamin B₁ powder (200 i.u. per g.) and the half-weekly doses were 0.03 and 0.04 g. alternately, all of them being diluted with finely ground dextrinized rice starch in the ratio 1:1.

The average increases in weight of the rats (♂ and ♀) are summarized in Table II.

Table II. *Responses of rats to doses of vitamin B₁ given (a) daily, and (b) half-weekly, the total amount given in 1 week being the same*

	Av. increase in wt. in 3 weeks (g.)	
	♂	♀
Daily dose	9.3 (3 rats)	13.7 (6 rats)
Half-weekly dose	32.5 (2 rats)	13.3 (7 rats)

Since *t* for the difference between the results from the male rats was 2.73, it is possible that the difference is significant but it is difficult to place any faith in a result obtained with only 2 or even 3 rats. The difference between the results from the female rats was almost nil and certainly insignificant, for *t* was found to be 0.27.

Fig. 3 shows the composite curves representing average increases in weight and amounts of food eaten by the different groups. From this chart may be seen:

(a) that in the preparatory period, the amount of food eaten increased during the first 10 days, remained at about 9 g. per day for 8 days and then decreased, the rate of increase in weight of the rats lessening only slightly a few days before doses of vitamin B₁ were given;

(b) that in the test period the male and female rats given daily doses and the female rats given half-weekly doses increased in weight to about the same amount; the 2 male rats given half-weekly doses grew much more, 21 and 44 g. respectively, but as noted above, the difference between this group and the group of male rats given daily doses could be regarded as insignificant;

(c) the slow increase in weight was not accompanied by an increase in the amount of the food eaten; the results suggested that the amount of food eaten was correlated with the rate of increase in weight rather than with the absolute weight of the rat at any time;

(d) as in the first experiment, no sudden increase in weight or in food eaten followed immediately after giving the large half-weekly doses of vitamin B₁;

(e) in the growth curves of individual rats (not published) it was noted that fluctuations in weight in the different rats were almost parallel from day to day.

Fig. 3. Composite curves of increase in weight and daily intake of food of rats which were given a diet deficient in vitamin B₁ only and were then given doses of a vitamin B₁ adsorbate (a) daily, or (b) the same total amount in half-weekly doses. Dose of vitamin B₁ sufficient to maintain life and to bring about an increase in weight of the rats.

This suggested the existence of undetermined external influences on the increases in weight, since all the rats were weighed early in the morning before giving them a fresh supply of food. Tourtelotte & Bacon [1935] found that temperature influenced the response of rats to vitamin A. It is possible that it also influences their response to other vitamins.

The experiment with crystalline vitamin B₁

This was carried out in the same way as the two experiments on the adsorbate, the dose of crystalline vitamin B₁ chosen being 5γ daily. The result may be seen in Table III.

Table III

	Av. increase in wt. in 3 weeks (5 rats in each of the 4 groups)	
	♂	♀
Daily	26.0 ($\sigma = 5.8$)	17.6 ($\sigma = 5.3$)
Half-weekly	15.4 ($\sigma = 10.9$)	9.6 ($\sigma = 8.3$)

This appears to show that giving doses daily is more effective than giving doses half-weekly, but since the value of t (the ratio of the difference between the average results to the square root of the sum of the squares of the standard errors of those results) was 1.9 for the male rats and 1.8 for the female rats, this difference cannot be regarded as significant.

SUMMARY

In a preliminary test it was found that equal supplements of the vitamin B complex produced equal increases in weight in groups of rats whether the doses were given daily or half-weekly.

In two separate experiments with (a) a small, and (b) a larger dose of a vitamin B₁ powder, equal supplements again produced equal responses whether given daily or half-weekly, except in 2 groups of only 2 and 3 males respectively; the difference between the results from these 2 groups was shown to be insignificant.

Records of amounts of food eaten were made daily. No sudden increase in weight or increase in food eaten was observed after giving the large half-weekly doses of vitamin B₁.

In an experiment with doses of crystalline vitamin B₁ given in aqueous solution, daily doses produced greater increases of weight than half-weekly doses but the difference was not significant.

It is concluded that, in determinations of vitamin B₁, doses of the standard or of the substance under examination may be given half-weekly instead of daily in order to save much time and labour.

REFERENCES

- Bacharach, Allchorne & Glynn (1936). *Biochem. J.* **30**, 2004.
Coward & Key (1934). *Biochem. J.* **28**, 870.
Tourtellotte & Bacon (1935). *J. Nutrit.* **10**, 683.