CCXXXIV. THE DETERMINATION OF THE ANTINEURITIC VITAMIN.

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PART I. PIGEONS.

THE methods employed at the present time for the estimation of the antineuritic vitamin are varied. Pigeons have long been used in tests in which the vitamin is administered (*a*) to prevent head retraction, (*b*) to cure head retraction and (*c*) to maintain body weight. Recently Kline *et al.* [1932] have described the use of chicks. Rats have been used in growth tests and in tests in which paralytic symptoms are cured [Smith, 1930]. Mice have also been recommended by Freudenberg and Cerecedo [1931] for growth tests.

Since the number of different factors discovered to be present in the commoner sources of the antineuritic vitamin continues to increase, methods of estimation in which an antineuritic effect is produced have an advantage over tests depending on growth or weight maintenance. Further an element of doubt remains whether the principle which cures the symptoms of paralysis in rats is identical with the antineuritic principle of Eijkman. It is true that Smith [1930] found that concentrates prepared by Seidell which prevented the onset of symptoms in pigeons were able to cure the symptoms in rats, but the ratio between the pigeon maintenance dose and the curative rat dose was not the same in different preparations. We feel that the claim of a method, in which birds are not used, to measure the antineuritic vitamin must rest ultimately on evidence that results obtained by that method are parallel with results obtained by a method in which birds are used.

Tests on pigeons must in our opinion be curative tests; as Smith has pointed out, a preventive test such as that of Williams [1916; 1917] takes too long, and a test like that of Seidell [1922], in which maintenance of body weight is the criterion, cannot be assumed to be a test for the antineuritic vitamin. It has, however, been difficult to find a curative test which gives trustworthy results. Kinnersley *et al.* [1928] have discussed the curative test in detail and base their procedure on the view "that there is fundamentally a relation between the amount of the factor supplied and the time of protection after cure."

When a pigeon develops head retraction after being fed on polished rice, Kinnersley *et al.* administer a dose of the preparation to be tested; if no relief of symptoms is seen, they give a further dose; they observe the number of days for which the pigeon remains cured. If the total dose of the preparation administered is x g., and if the duration of the cure is y days, they express the potency of the preparation as y/x "day doses per g." The average result from several birds is taken as the true result.

As the work in this laboratory involves many routine examinations, we have been attracted to the curative test for pigeons because of the simplicity of the test; it involves less work and skilled attention than the rat test described by Smith, or indeed than any other vitamin test. We have accordingly investigated the response of pigeons suffering from head retraction to different doses of several preparations containing vitamin B_1, testing the effect of each dose of a preparation upon several pigeons. The investigation has been continued during 31 months in which 688 birds have been given a diet of polished rice and water; as a result we are proposing a different method of using pigeons which we believe makes it possible for more accurate results to be obtained than are given by the day-dose method in routine tests.

Details of the treatment of the pigeons.

The pigeons were obtained from a dealer in groups of 30 at one time and were birds weighing 300–450 g. We did not give them a stock diet for a preliminary period but fed them on polished rice at once; nor did we wash the rice. Out of the total number of 688 birds used by us, head retraction occurred in 310 (45 %). The figures for different months are given in Table I and show

Table I. *Apparent seasonal variation in percentage of pigeons developing head retraction on a polished rice diet.*

	Total no. of birds given polished rice diet			Percentage of birds in which symptoms occurred in 30 days		
	1931	1932	1933	1931	1932	1933
January	30	—	32	23	—	19
February	30	32	32	40	42	69
March	23	32	30	40	53	60
April	32	30	—	50	47	—
May	32	24	30	41	37	57
June	32	—	—	56	—	—
July	20	—	24	45	—	37
August	—	—	—	—	—	—
September	31	—	—	55	—	—
October	32	32	—	53	47	—
November	32	32	—	59	34	—
December	32	32	—	28	42	—

that the proportion was lower in January, when the weather was cold. When head retraction was seen in a pigeon, the bird had usually lost from 70 to 90 g., though occasionally as little as 50 or as much as 110 g. Birds which did not show head retraction in 30 days were not used.

The pigeons were kept in the open air in cages with wire netting screens of ½-inch diameter to prevent access to faeces. When a pigeon showed head retraction, it was taken to the laboratory, the dose was given by mouth, and without delay it was put again in the open air in a separate cage. Attention was not paid to symptoms other than those of typical head retraction. The pigeon was then observed at intervals and its condition recorded at the end of each period of 24 hours from the time the dose was given. The pigeon was observed for several days to see for how long it remained free from symptoms. If head retraction had fully disappeared at the end of 24 hours, but had reappeared by 48 hours, the result was recorded as a cure for 1 day. Freedom from symptoms for 48 hours, but for less than 72 hours, was recorded as a cure for 2 days, and so on.

If a pigeon was not cured within a few hours of the administration of the dose, no further dose was given. All doses were given in about 5 cc. of water, half of the water being kept to wash down traces. A pipette fitted with a short rubber tube of 2 mm. internal diameter was used.

Preparations examined.

The preparations examined were (1) a sample of dried yeast supplied by the National Institute for Medical Research for an investigation undertaken as a preliminary to the International Conference in London, 1931; (2) a sample of activated acid clay prepared by Prof. Jansen of Amsterdam and supplied by the National Institute for Medical Research; (3) a second sample of acid clay proposed for use as the International Standard of the antineuritic vitamin, also supplied by the National Institute for Medical Research; (4) two concentrated solutions kindly given to us by Prof. Peters; (5) a commercial extract of rice polishings; (6) a commercial soft extract of yeast.

These six preparations appeared to us to provide a sufficiently diverse assortment of preparations of the antineuritic vitamin.

Experimental results.

It will be convenient to describe some results relating to the method of estimating potency used by Kinnersley *et al.* As already stated, the percentage of pigeons which we have observed to develop head retraction within 30 days when fed on polished rice is 45. Prof. Peters has kindly informed us that the percentage observed in his laboratory is almost the same. We suggest that the close correspondence between these percentages means that certain precautions taken in Prof. Peters's laboratory are unnecessary (*e.g.* feeding the pigeons on a uniform stock diet for several days before giving them the rice diet and washing the rice). The omission of these steps means a saving of time and expense in routine work.

The relation between dose and duration of cure.

We have examined the conclusion of Kinnersley *et al.* that there is a relation between the amount of the antineuritic factor supplied and the time of protection after cure, and the results are given in Table II.

The preparations examined were each administered in one or more doses, and the average duration of cure was calculated for each selected dose. Thus the average duration of the cure of 11 birds to which 0·03 g. of the original sample of acid clay was administered was 3·7 days; hence by Peters's method the potency was 123 day doses per g. In calculating the average duration of cure, no account was taken of birds to which the dose was administered and which were not cured. The final column in Table II gives the potency of each substance expressed as units per g. or per cc., taking the unit as the activity present in 0·01 g. of the standard acid clay. The different figures for the potency of each substance should of course be alike if the day-dose method of comparing the activity of the different preparations with the standard is satisfactory. Inspection shows that the different figures are by no means alike. The potency of the original sample of acid clay was found to be 357, 176, 104 and 91 units per g. To the first of these figures less attention can be paid since it is based

Table II. *Vitamin B_1 potency of various substances estimated in terms of the International Standard by day-dose method.*

Material	Dose	No. of birds cured	Average duration of cure (days)	Day doses per g. or cc.	Potency in terms of Standard units per g. or cc.
Acid clay (original sample)	0·015 g.	4	3·75	250	357
	0·03 g.	11	3·7	123	176
	0·06 g.	16	4·4	73	104
	0·12 g.	13	7·7	64	91
Acid clay (Standard)	0·03 g.	7	2·1	70	100
Dried yeast	0·031 g.	2	4·5	145	207
	0·062 g.	5	1·8	28·8	41
	0·125 g.	15	4·4	35·2	50
	0·25 g.	16	5·9	23·6	34
Vitamin B_1 solution (*a*)	0·1 cc.	4	2·0	20	29
(*b*)	0·2 cc.	6	1·8	9	13
(*a*)	0·25 cc.	7	2·1	8	11·4
(*b*)	0·35 cc.	9	2·5	7	10
Extract of rice polishings	0·25 cc.	5	1·6	6·4	9
	0·5 cc.	10	5·1	10·2	14
	1·0 cc.	10	5·7	5·7	8
	2·0 cc.	11	7·5	3·75	5·3
Yeast (soft extract)	0·12 g.	3	1·7	14	20
	0·24 g.	6	3·5	14	20

Note. The vitamin B_1 solutions (*a*) and (*b*) were two different tubes kindly supplied by Prof. Peters, which were probably, but not certainly, alike in potency. Since examination showed no difference between them, they have been tabulated as one preparation.

on a cure of only 4 birds; but there is no explanation of the difference between the estimate of 176 units per g. and the other estimates of 104 and 91 units per g., save that the error of the method is large, or that the relationship between duration of cure and dose of vitamin B_1 given is not a straight line. (See the section on the statistical examination of these results.) The potency of the extract of rice polishings was found to be 9, 14, 8 and 5·3 units per g., the highest value being nearly three times the lowest.

In spite of these divergences there are some similarities; the two values 104 and 91 for the original sample of acid clay are close together; the highest and lowest of the three figures for dried yeast, 41, 50 and 34 do not differ by more than 50 %; the three figures 13, 11·4 and 10 for the vitamin B_1 solution are close together; two of the figures for the extract of rice polishings are respectively 8 and 9, while the two figures for the soft extract of yeast are identical.

We draw the conclusion that there is certainly foundation for the view of Kinnersley *et al.* that a relation exists between the size of the dose of vitamin B_1 administered and the duration of the cure, but that, when groups of 10 or 15 birds are used for an estimation, errors of 50 or 100 % may occur. It will be observed that the potency in day-doses per g. or per cc. of the materials in Table II diminished as the dose which was examined grew larger; it follows that the potency of any unknown material expressed as day doses per g. will depend to some extent on the dose which is chosen, becoming lower as the dose becomes higher. It is right to point out that these comments apply only to experiments in which different pigeons are used for examining the effect of different doses. If the same group of pigeons had been used for testing the effect of each of the doses of the different substances mentioned in Table II, it is possible that we would have found that the average duration of cure was proportional to the dose; Kinnersley *et al.* [1928] have published some evidence in favour of this expectation. For routine purposes, when a preparation is to be compared with the International Standard, the use of the same group of pigeons for both standard and unknown preparations would necessitate a long delay in the completion of a test.

The percentage of birds cured.

Apart from the attempt of Kinnersley *et al.* to estimate potency in terms of "day-doses," other workers have determined potency by finding the minimum curative dose. The difficulty of doing this arises from the variation in the response of different pigeons; head retraction is not cured in some birds by a dose of the vitamin which will satisfactorily cure others, and in practice it is impossible to define what is meant by a "minimum curative dose." It is tempting to explain differences of this kind by supposing that at the time head retraction occurs the deficient diet has left one bird less able to absorb the vitamin than another; but such explanations do not help to overcome the practical difficulty of estimation. Similar variations in the response of animals to drugs are well known in pharmacology, and Trevan [1927] showed how they were to be overcome. We have followed the principles demonstrated by Trevan in the following experiment.

The sample of acid clay first supplied by the National Institute for Medical Research and the sample of dried yeast were tested in different doses to determine the proportion of birds cured by each dose. Thus the dose of 0·015 g. acid clay was given to each of 16 pigeons with head retraction; 4 were cured and remained cured for at least 24 hours; the dose of 0·03 g. acid clay was given to each of another group of 16 birds, of which 8 were cured. The results are recorded in Table III. It will be seen that as the dose either of acid clay or of dried yeast was increased, the percentage of birds cured also increased. In

Table III. *Vitamin B_1 potency of various substances estimated in terms of the I.S. by the percentage of birds cured.*

Material	Dose	No. of birds given dose	No. cured	% cured	Units per g. or cc.	Weighted mean (units)
Acid clay (original sample)	0·015 g.	16	4	25	118	110
	0·03 g.	23	11	48	100	
	0·06 g.	20	16	80	115	
	0·12 g.	20	13	65	—	
Acid clay (Standard)	0·03 g.	15	7	47	100	100
Dried yeast	0·031 g.	16	2	12·5	32·1	33·8
	0·062 g.	16	5	31	35·7	
	0·125 g.	20	15	75	39·8	
	0·250 g.	20	16	80	27·7	
Vitamin B_1 solution (*a*)	0·1 cc.	13	4	31	22·1	16·2
(*b*)	0·2 cc.	10	6	60	17·8	
(*a*)	0·25 cc.	13	7	54	13·1	
(*b*)	0·35 cc.	13	9	70	12·4	
Extract of rice polishings	0·25 cc.	8	5	62	14·8	17·5
	0·5 cc.	12	10	83	24·6	
	1·0 cc.	12	10	83	12·3	
	2·0 cc.	12	11	92	—	
Yeast (soft extract)	0·12 g.	10	3	30	17·9	19·6
	0·24 g.	8	6	75	20·7	

Fig. 1 the percentage of birds cured has been plotted against the dose, and the points for the acid clay and for the dried yeast fit curves of the usual sigmoid shape. The point indicating the result of administering the largest dose of acid clay lies off the curve through the other points; it was observed that all the birds which were not cured by this dose were dead on the day after the dose was given. This unusual occurrence gave us the impression that the curative action of the large dose was complicated by a toxic effect.

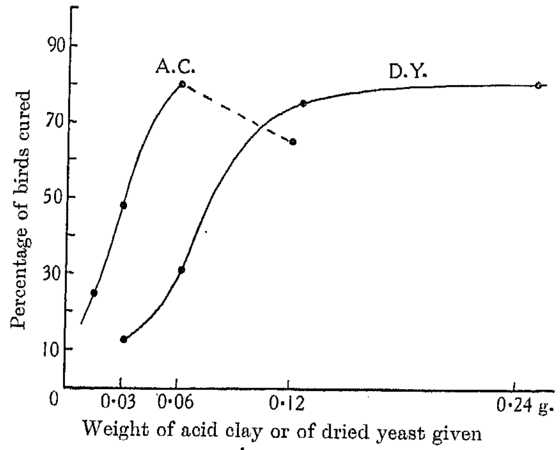

Fig. 1. A.C., Curve of response of polyneuritic pigeons to graded doses of activated acid clay (Jansen's preparation of vitamin B_1). D.Y., Curve of response of polyneuritic pigeons to graded doses of dried yeast.

From the two curves shown in Fig. 1 it is possible to make a quantitative comparison between the potency of the dried yeast and that of the acid clay. Thus a line parallel with the abscissa may be drawn to cut the curves at different points, and the ratio of the abscissae determined. Thus such a line drawn through the ordinate corresponding to 30 % cuts the acid clay curve at a point of which the abscissa is 0·02, and cuts the dried yeast curve at a point of which the abscissa is 0·06. The ratio of the potency of dried yeast to acid clay is, therefore, 0·02/0·06, or 0·33. Similarly the ratio of the potency determined by a line drawn through the ordinate corresponding to 50 % is 0·39, and that determined by a line drawn at 70 % is 0·42. The three figures 0·33, 0·39 and 0·42 give an average of 0·38.

The estimation of unknown preparations.

While it is true that a comparison of the potency of dried yeast with that of acid clay has been obtained by the foregoing experiments, it may be argued that the number of pigeons needed to construct a curve for an unknown preparation like the curve in Fig. 1 is too great for the method to be of practical value. The testing of unknown preparations can be abbreviated, however, in the following way. We have constructed from the two curves in Fig. 1 a third curve shown in Fig. 2, which is an approximation to the characteristic curve [see Trevan, 1927] for the response of pigeons with head retraction to doses of the antineuritic vitamin. The ordinates in Fig. 2 are, as in Fig. 1, the percentage of pigeons cured 24 hours after administration of the dose. The abscissae are doses expressed in arbitrary figures. The abscissa corresponding to the cure of

50 % of birds is given the value 1·0. Half-way between this point and the origin is 0·5, *etc.* The points on the acid clay curve in Fig. 1 are points corresponding to the cure of 25, 50 and 80 % of pigeons; if the dose curing 50 % be taken as 1·0, then the abscissae of these points in Fig. 2 are 0·5, 1·0 and 2·0, for the doses were in this proportion. Similarly the points on the dried yeast curve are plotted in Fig. 2 again taking the dose (determined by interpolation in

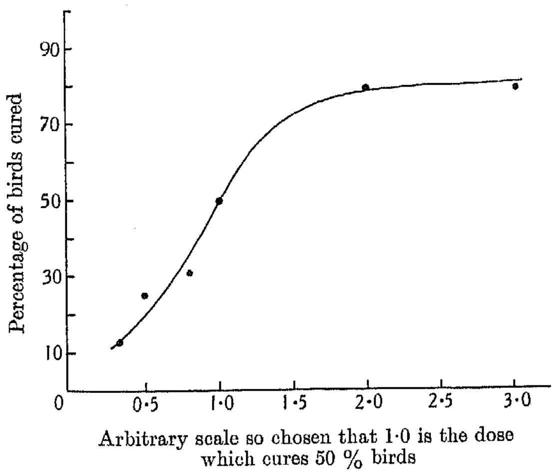

Fig. 2. Curve of response constructed from results of tests on acid clay and on dried yeast.

Fig. 1) which cures 50 % of pigeons as 1·0. The points on the two curves in Fig. 1, adjusted in this way, fit one curve remarkably well, the coincidence supporting the view that the same phenomenon is responsible for the shape of both curves in Fig. 1.

The curve in Fig. 2 can now be used for the estimation of unknown preparations of the vitamin, and the manner of its use is most simply explained by a comparison which was made of the two water clear solutions containing vitamin B_1, prepared by Prof. Peters, with the International Standard acid clay.

Comparison of vitamin B_1 solution with the Standard.

The vitamin B_1 solution was administered in a dose of 0·1 cc. to each of 13 birds suffering from head retraction; 4 birds were cured (approx. 30 %). The International Standard acid clay was administered to 15 birds suffering from head retraction in a dose of 0·03 g.; 7 birds were cured (approx. 50 %). The relative potency of doses curing 30 and 50 % of birds is given by Fig. 2 as 0·65 to 1·0.

Hence
$$\frac{0\cdot1 \text{ cc. vitamin } B_1 \text{ solution}}{0\cdot03 \text{ g. Standard}} = \frac{0\cdot65}{1\cdot0}$$

or
$$1 \text{ cc. vitamin } B_1 \text{ solution} = 0\cdot2 \text{ g. Standard} = 20 \text{ units.}$$

A further examination of the vitamin B_1 solution was made in which 0·25 cc. was administered to each of 13 birds, of which 7 were cured, or approximately 50 %; hence 0·25 cc. vitamin B_1 solution was found approximately equivalent to 0·03 g. Standard, which contains by definition 3 units; hence 1 cc. = 12 units.

No further observations were possible with this material, as very little was left, but Prof. Peters kindly sent us another tube of solution which he believed to be similar to the first tube, though he was not certain. The small remainder of the first tube was mixed with this second tube, and the mixture was administered to pigeons in the two doses 0·2 and 0·35 cc. The first dose cured 6 out of 10 birds, indicating a potency of 17·5 units per cc., and the second dose cured 9 out of 13 birds, indicating a potency of 12 units per cc.

Comparison of the day-dose and percentage-cure methods.

The results obtained with the two solutions supplied by Prof. Peters made possible a useful comparison shown in Table IV of the day-dose method of calculating potency and the percentage-cure method. For this comparison the

Table IV. *Examination of vitamin B_1 solution.*

First tube, A; second tube, B.

	Units per cc.			
Dose (cc.)	Day-dose method	Average	Percentage-cure method	Average
0·1 A	29	15·9	20	15·4
0·2 B	13		17·5	
0·25 A	11·6		12	
0·35 B	10		12	

two solutions examined were assumed to be identical in potency; the results indicated that they were. The results by the day-dose method have already been given in Table II.

Observations on other preparations.

The extract of rice polishings and the soft extract of yeast were also examined by the percentage-cure method. In a dose of 0·25 cc. the extract of rice polishings cured 5 out of 8 pigeons; in a dose of 0·12 g. the soft extract of yeast cured 3 out of 10 pigeons, while a dose of 0·24 g. cured 6 out of 8 pigeons. From these results the potency in units per cc. or per g. has been calculated and expressed in Table V.

Table V. *Comparison between results obtained by the day-dose and percentage-cure methods.*

Material	Dose	Potency by day-dose method (units per g. or cc.)		Potency by percentage-cure method (units per g. or cc.)	
Acid clay (original sample)	0·03 g.	176	123	100	
	0·06 g.	104			
	0·12 g.	91			
Dried yeast	0·062 g.	41	42	34	
	0·125 g.	50			
	0·25 g.	34			
Extract of rice polishings	0·25 cc.	10·8		12	
Yeast (soft extract)	0·12 g.	20	20·5	17	18·5
	0·24 g.	21		20	

The agreement seen in Table V between the average figures for the potency by the day-dose method and for the potency by the percentage-cure method is striking; for the original sample of acid clay the agreement would be close but for the one high estimation by the day-dose method. The average figures for the two methods differ by 20 % for dried yeast, by 11 % for the extract of rice polishings, while the means of the two pairs of results for the soft extract of yeast differ by 10 %. The two methods have given the same average results in these experiments because several doses were tested, and the high values calculated from the low doses in the day-dose method balanced the low values calculated from the high doses.

Non-specific cures.

The pigeon curative method has been criticised because some other substances than the antineuritic vitamin will give temporary relief from head retraction in some birds.

Two questions appear to us to be confused in the minds of those who offer this criticism, namely whether a substance contains any vitamin B_1, and how much vitamin B_1 it contains. In detecting the presence of any vitamin B_1, it is obviously important to watch for non-specific effects; on the other hand, in determining the potency of a substance known to contain vitamin B_1, it is less important, since the substances which produce non-specific cures can usually be assumed to be absent. Histamine, pilocarpine, nitrites, thyroxine and choline [Dutcher, 1919; Abderhalden, 1923; Peters, 1924] are not present in extracts of yeast or rice polishings in sufficient quantity to interfere with the estimation of the vitamin B_1.

Peters adopts the precaution of administering dextrose to each bird which develops head retraction; we have, therefore, examined the effect of giving 0·05 g. dextrose in 5 cc. water to a series of pigeons prepared here. Out of 16 birds to which the dose was administered, 2 were cured for periods of 24 and 48 hours respectively. It follows from this result, that if we were examining a substance of unknown origin, in which dextrose might be present, it would be wrong to calculate the amount of vitamin from the cure of only a small proportion of birds; it would be necessary to test the effect of a larger dose. It is of course wise to do this whatever the origin of the preparation being tested; thus, in testing the soft extract of yeast as already described, the potency was first calculated from the cure of 3 birds out of 10 by a dose of 0·12 g.; a further estimate was then made by administering twice the dose, when 6 out of 8 birds were cured. The final estimate of 18·2 units per g. did not differ greatly from the first estimate of 16·6 units per g.

Birds cured for 1 day.

The definition of a cure of head retraction given earlier in the paper was that a bird should be found free from symptoms at the end of 24 hours after the dose was administered. If a bird found to be cured at that time developed symptoms again 48 hours after the first sign of head retraction, the cure was counted as a cure for 1 day. The opinion was expressed to us that it was unsafe to assume that a cure for less than 48 hours was a genuine cure. We have, therefore, recalculated our results, excluding all birds in which the cure lasted for less than 48 hours. We have found, however, that the results so obtained did not differ appreciably from those already given in Tables II and III. Thus the potency of dried yeast became 47 units per g. instead of 42 units per g. when using the day-dose method of comparison and became 34 units per g. instead of 38 units per g. using the percentage-cure method.

Duration of head retraction before dosing.

When pigeons developed head retraction on the diet of rice, some were observed at the beginning of the day, and some were observed later; as no inspections were made during the night, birds found with head retraction in the morning may have had the symptoms for 15 hours before the dose was given, whereas birds found later in the day were given the dose within an hour or two of the onset. We have examined the records to see whether the birds in a group which were not cured by a particular dose were those which were found with symptoms in the early morning, and whether the birds which were cured were those found later. The examination showed no such correlation; the cure of head retraction by a particular dose was not dependent on the lapse of a short time between the onset of symptoms and the administration.

PART II. RATS.

The growth-promoting property of vitamin B_1.

It has been shown by many workers that vitamin B_1 (as distinct from vitamin B_2 or the vitamin B complex) is necessary for the growth of the rat. Sherman and Axtmayer [1927] demonstrated the supplementary nature of wheat germ and dried milk and concluded that this was due to the greater amount of vitamin B_1 in wheat germ than in dried milk. Chick and Roscoe [1927] found that an extract of the antineuritic vitamin prepared from yeast according to Peters's method would not promote growth in rats fed on a diet deficient in vitamin B (complex). If, however, this was supplemented by a daily dose of yeast autoclaved at 120° for 5 hours to destroy the antineuritic vitamin, growth was resumed. They showed also that rats lose weight and die more quickly on a shortage of vitamin B_1 than on a shortage of vitamin B_2. Hassan and Drummond [1927] showed that two factors in yeast, differentiated by their behaviour to alkalis, were necessary for the normal rate of growth of rats fed on high-protein diets. It then became generally recognised that, in order to estimate vitamin B_1 by means of its growth-promoting property, vitamin B_2 must be liberally supplied to the rats.

Aykroyd and Roscoe [1929], in estimations of vitamin B_2, gave a daily dose of 0·1 cc. of Peters's antineuritic concentrate (equivalent to 0·6 g. yeast) to their rats in addition to the vitamin B-free diet.

Finally, Chick and Roscoe [1929] developed a method for the estimation of vitamin B_1 in foodstuffs by means of its growth-promoting property. They supplied vitamin B_2 by a daily dose of autoclaved yeast (120° for 5 hours) or substituted the purified caseinogen of the diet by coagulated egg-white from fresh eggs, which they had shown to contain vitamin B_2. They discarded the latter method, however, as the growth of the rats was not maintained long enough for the test, and they concluded that autoclaved yeast contains a heat-stable factor necessary for growth and distinct from both vitamin B_1 and vitamin B_2. Fresh egg-white does not contain this factor.

Roscoe [1930; 1931, 1, 2] compared the vitamin B_1 content of various vegetables and fruits by determining the dry weight of each necessary to produce a given rate of growth in 5 weeks. Vitamin B_2 was given to each rat as a daily dose (equivalent to 0·5 g. dry yeast) of an extract made from washed brewer's yeast by boiling it with 0·01 % acetic acid and then autoclaving the extract for 5 hours at 120° (p_H *ca.* 5·0).

Halliday [1932] supplied vitamin B_2 as 15 % autoclaved yeast in the basal diet of rats which were used for estimations of vitamin B_1.

Guerrant and Dutcher [1932] used as a source of vitamin B_2 a dry autoclaved (6 hours, 15 lbs.) extract of yeast which had been precipitated at an 80 % alcohol concentration of the aqueous extract in the filtrate from 50 % alcohol concentration.

Chick and Jackson [1932], in estimating vitamin B_1 with the rat as the experimental animal, used as a source of B_2 a small daily ration of a watery yeast extract, autoclaved for 4 hours at 120° at p_H 5·0.

It then seemed to us that it would be useful to construct a curve of response relating average increase in weight of groups of rats to daily dose of vitamin B_1 given, as we had previously done for vitamin A and vitamin D.

I. *Diet of the rats.*

Young rats weighing 55–70 g. were given a diet consisting of:

	%
Caseinogen (light white B.D.H. not extracted) ...	15
Dextrinised rice starch	71
Agar-agar	2
Salt mixture (Steenbock's 40)	4
Autoclaved yeast...	8

Each rat was given 5 drops of a good sample of cod-liver oil twice a week to supply vitamins A and D.

The yeast was spread on tins in thin layers, 40 g. over 44 sq. in., and autoclaved for 6 hours at 15 lbs. additional pressure. It became dark brown in colour and moist. It was dried by leaving overnight in an electric oven whose current had been turned on until the temperature was raised to 100° and then turned off before the trays of yeast were put into it. The yeast was caked after drying and had to be ground in a mortar.

The rats were given this diet until they ceased to grow. During this preparatory period of 14 to 18 days, nearly every rat increased in weight by at least 10 g., many by 20 g. and some even by 30 g. Those which were given no vitamin B_1 after growth ceased, lost weight rapidly and died in 21–38 days from the beginning of the preparatory period.

The rats were kept 4 together in a cage during the preparatory period, on grids of 0·3 in. mesh. During the giving of doses, each rat was kept in a separate cage on a grid of 0·5 in. mesh.

Twelve litters of 5 or 6 rats each were used for testing 5–6 doses of acid clay, each rat of a litter receiving a different dose of acid clay. The doses tested were 0, 0·005, 0·01, 0·02, 0·04, 0·1 g. For the first 6 litters each dose was weighed daily and mixed with a little water in a separate dish for each rat. Some of the rats refused to eat their doses completely but when the dose was mixed with a little dextrin it was always eaten. For the later work, the acid clay was mixed at once with dextrin (1 part acid clay to 4 or to 9 parts dextrin as convenient) and the diluted material used for all the rats.

II. *First curve of response to graded doses of activated acid clay, the International Standard of reference for vitamin B_1 (8 % autoclaved yeast in the diet).*

No rats developed polyneuritis or convulsions [Smith, 1930] during this experiment, whether they received a dose of acid clay or not.

The growth response of the rats was graded to the dose of acid clay given. The male rats made greater increases in weight on each dose (but one) than the female rats (Fig. 3), but the difference was less than the difference in similar experiments with vitamins A and D [Coward *et al.*, 1931; 1932]. The mean increases in weight of the bucks and does respectively have themselves been averaged for constructing a curve of response for use in interpreting estimations on other substances. The results of the tests on other substances have been treated similarly for the sake of simplicity.

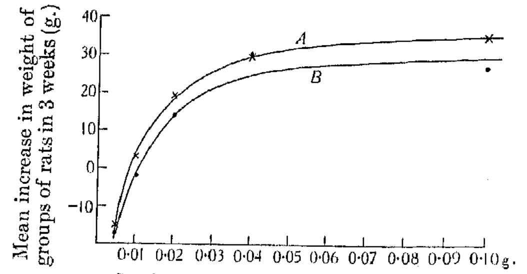

Fig. 3. Curves of response of groups of rats to different doses of activated acid clay (I.S. for vitamin B_1 estimations). Each rat of a group received the same daily dose for 3 weeks. The basal diet contained only 8 % autoclaved yeast as a source of vitamin B_2.

A, determinations from male rats. *B*, determinations from female rats.

In the first 5 litters we used for the construction of this curve, several of the rats given the highest dose of acid clay (0·04 g.) grew less quickly than was expected from the growth of the rats on the lower doses. We, therefore, used one rat of each of the later litters for testing a dose of 0·1 g. acid clay. The response of the bucks to this dose was higher than to the dose of 0·04 g. but that of the does was slightly less. This, together with results of tests of other substances made at the same time, led us to the conclusion that the basal diet we were using was deficient in some substance or substances other than vitamin B_1. We suspected the destruction of vitamin B_4 in the autoclaving of the dried yeast. We were able to examine this possibility through the kindness of Dr V. Walker (Reader) who gave us sufficient solution of vitamin B_4 to dose 12 of our rats receiving different doses of acid clay, but growing very slowly. This did not, however, increase the rate of growth of any one of our rats, and we concluded that the diet was not lacking in vitamin B_4. We then suspected a deficiency of vitamin B_2 or some other heat-labile factor in the basal diet. Tests on other substances (Section III) and the construction of a fresh curve from rats given a basal diet containing 20 % autoclaved yeast (Section IV) confirmed this view.

III. *Comparison of other substances with activated acid clay with regard to their vitamin B_1 potency.*

Four substances were examined for their content of vitamin B_1 at the same time that the curve of response to doses of activated acid clay was being constructed. The substances were (*a*) a sample of dried yeast (used by several laboratories in connection with preliminary work on the International Standard), (*b*) a sample of wheat embryo (also used by the same laboratories for the same work), (*c*) a commercial sample of a food and (*d*) a commercial sample of a yeast extract (I). Each was tested in two different doses. It happened that the lower dose of each substance gave a result comparable with the result on a low dose of acid clay, but the higher dose of each substance gave a result considerably higher than that given by the highest dose of acid clay. The mean increase in weight produced by each of the higher doses was well above the curve. In Fig. 4 the mean increase in weight of the animals given the lower

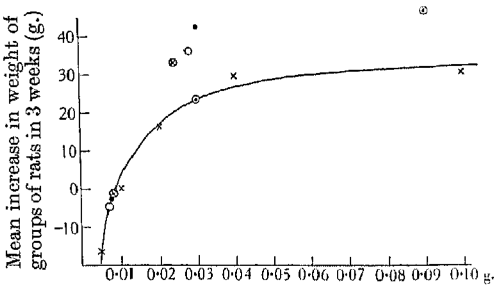

Fig. 4. Uselessness of this curve of response to estimate the vitamin B_1 content of other substances. (Each point is the average of the mean increases in weight of the bucks and does respectively, *i.e.* no point is overweighted by an excess of results from bucks.)

× = Mean increases in weight of the groups of rats given graded doses of the Standard.

• = Mean increases in weight of groups of rats given daily 0·05 and 0·2 g. respectively of a sample of dried yeast I.

⊙ = Mean increases in weight of groups of rats given daily 1·0 and 3·0 g. respectively of a commercial food sample.

⊗ = Mean increases in weight of groups of rats given daily 0·1 and 0·3 g. respectively of a sample of wheat embryo.

○ = Mean increases in weight of groups of rats given daily 0·05 and 0·2 g. respectively of yeast extract I.

The result from the lower dose of each test was plotted on the curve itself. The result from the higher dose of each test was plotted against the abscissa corresponding to the appropriate multiple of the abscissa found for the lower dose.

dose of the substance was marked on the curve, the abscissa of that point noted, and the mean increase in weight of the animals given the higher dose was plotted against the abscissa which bore the same ratio to the first abscissa that the higher dose bore to the lower dose of substance tested. It was obvious that the dried yeast, the wheat embryo, the yeast extract and the food substance each contained some substance necessary for growth which was not supplied in sufficient amounts by our basal diet *plus* large doses of acid clay.

We, therefore, decided to repeat the experiment using 20 % autoclaved yeast in the diet which was then made up as follows.

	Parts
Caseinogen (light white, B.D.H. untreated)	15
Dextrinised rice starch	79
Agar-agar	2
Salt mixture (Steenbock's 40)	4
Autoclaved yeast...	25

The same amount of cod-liver oil was given to each rat as before.

IV. *Second curve of response relating mean increase in weight of groups of rats to dose of vitamin B_1 given (20 % autoclaved yeast in basal diet).*

A second curve of response was constructed by the same method as the first, but 20 % yeast autoclaved under apparently the same conditions was used instead of 8 %. Four litters of rats were used for this experiment. The curve of response was steeper than the first (Fig. 5). It justified our conclusion that in the former experiment we had not given a large enough percentage of autoclaved yeast in the basal diet.

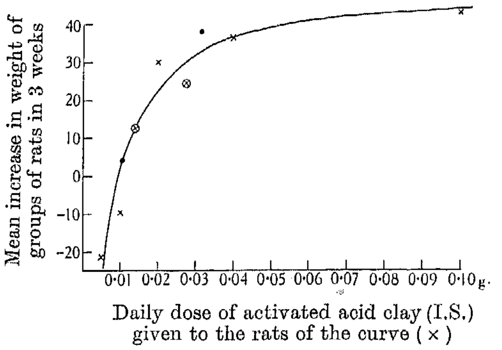

Fig. 5. Curve of response of groups of rats to graded doses of activated acid clay (I.S. for vitamin B_1 estimations). Each rat of a group received the same daily dose for 3 weeks. The basal diet contained 20 % autoclaved yeast as a source of vitamin B_2.

Usefulness of this curve for estimating the vitamin B_1 content of other substances.

× = Mean increases in weight of groups of rats given graded doses of the Standard.

• = Mean increases in weight of groups of rats given daily 0·05 and 0·15 g. respectively of dried yeast.

⊗ = Mean increases in weight of groups of rats given daily 0·1 and 0·2 g. respectively of a yeast extract II.

The results were plotted as in Fig. 4.

V. *Confirmation of the validity of the second curve of response relating mean increase in weight of groups of rats to dose of vitamin B_1 given.*

Two substances, dried yeast II and a yeast extract II (both different from those used previously), were tested in 2 doses each. The mean increases in weight of the 2 groups of rats given the lower doses of the 2 substances were plotted on the curve, and the mean increases in weight of the rats given the higher doses were plotted against the abscissae which bore the same ratios to the abscissae of the lower doses as the ratios of the actual doses. These points fell very nearly on the curve of response, one somewhat above the curve, the other somewhat below (Fig. 5). Thus the second curve appears to be a better one than the first in that, so far as it has been tested, the responses do not seem to be limited by an insufficiency of any factor in the basal diet. The response to a test substance when this diet is used appears to be a response to vitamin B_1 only.

VI. *The possible logarithmic nature of the curves.*

Each of the two curves described in this paper was constructed from 5 determinations of the mean increases in weight in 3 weeks of 5 groups of rats, the rats of each group being given daily doses of 0·005, 0·01, 0·02, 0·04 and 0·1 g. International Standard preparation of vitamin B_1. The curves were drawn as smoothly as possible near the points plotted on graph paper. Neither of the curves so drawn was logarithmic, and the best logarithmic curve through either set of 5 points (determined from the best straight line through the points obtained by plotting mean increases against the logs of the doses) did not fit the actual points at all well. But a logarithmic curve fitted the four lower points of the first set very well and another fitted those of the second set only rather less well (Fig. 6). This is similar to our finding in our vitamin A work, in which

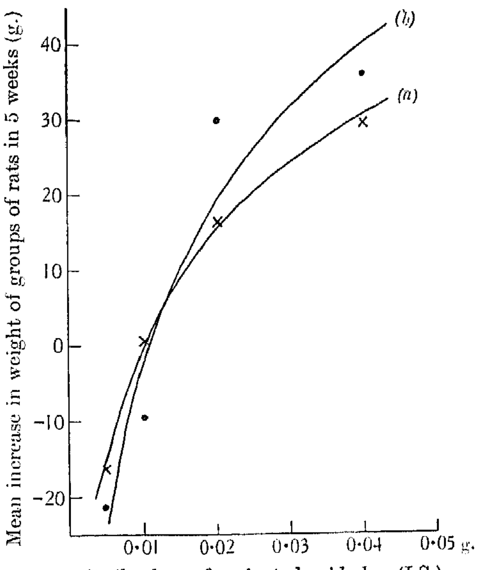

Fig. 6. The best logarithmic curves relating the mean increase in weight of groups of rats to dose of vitamin B_1 given when the basal diet contains (*a*) 8 % and (*b*) 20 % respectively of autoclaved yeast as a source of vitamin B_2.

a logarithmic curve fitted the responses of rats (mean increases in weight in 5 weeks) to doses of 0·25, 1·0, 1·5, 2·5, 7·5 mg. respectively of cod-liver oil, but not the response to the highest dose, 20·0 mg. It should, however, be safe to accept the logarithmic equations for the curves for the range of mean increases which determined the shapes of the curves.

The equation for the first vitamin B_1 curve reported in this paper (Fig. 6 (*a*)) for the range -16 to $+30$ g. mean increase in weight is $y = 102{\cdot}3 + 51{\cdot}1 \log x$.

The equation for the second curve reported in this paper (Fig. 6 (*b*)) for the range -21 to $+36$ g. mean increase in weight is $y = 139{\cdot}4 + 70{\cdot}4 \log x$.

VII. *Justification for the acceptance of the logarithmic equations for the curves of response.*

The logarithmic curve through the first 4 points in the first experiment fits those points so well that it can be accepted without question as representing the relation between response and dose of vitamin B_1 given for the range of doses 0·005 to 0·04 g. I.S. Its equation is $y = 102{\cdot}3 + 51{\cdot}1 \log x$.

The logarithmic curve through the first 4 points in the second experiment fits those points less well and it might be accepted with some hesitation. When, however, the results of feeding 2 different doses of each of 2 different substances were interpreted by means of this curve, the ratios of the apparent potencies of the respective pairs of doses were so nearly the known ratios of the doses that we feel no hesitation in accepting the curve with its logarithmic equation as representing the relation between the mean increase in weight of a group of rats (all given the same dose) and the dose of vitamin B_1 given (Table VI). Its equation is $y = 139\cdot4 + 70\cdot4 \log x$.

Table VI. *Confirmation of validity of second curve of response relating mean increase in weight in 3 weeks of groups of rats to dose of vitamin B_1 given (I.S.).*

Substance tested	Daily dose (g.)	No. of rats	Mean increase in wt. in 3 weeks (g.)	Abscissa of curve corresponding to the increase in weight	Ratio of potencies of doses according to ratio of abscissae	Actual ratio of doses
Dried yeast II	0·05	3	4·2	0·0125	1 : 2·9	1 : 3
,,	0·15	4	38·0	0·0365		
Yeast extract II	0·1	4	12·8	0·0160	1 : 1·6	1 : 2
,,	0·2	4	24·5	0·0255		

VIII. *Interpretation, by means of the second curve, of results which could not be interpreted by means of the first curve.*

In Table VII are collected details of the tests of the four substances mentioned in Section III, which could not be interpreted by means of the curve of

Table VII. *Interpretation, by means of the second curve, of results which could not be interpreted by means of the first curve.*

Substance tested	Dose (g.)	No. of rats ♂	No. of rats ♀	Mean increase in wt. in 3 weeks (g.) ♂	Mean increase in wt. in 3 weeks (g.) ♀	Average mean increase of ♂ and ♀ rats	Interpretation by second curve of response. Diet contained 20 % autoclaved yeast: Abscissa corresponding to mean increase in weight	Interpretation by second curve of response. Diet contained 20 % autoclaved yeast: Apparent ratio of doses	Actual ratio of doses
Dried yeast I	0·05	6	4	−1·3	−4	−2·65	0·0096	1 : 4·2	1 : 4
	0·2	7	3	49·7	36·0	42·85	0·0407		
Food substance	1·0	3	3	23·3	23·7	23·5	0·0226	1 : 2·1	1 : 3
	3·0	3	3	55·3	37·6	46·45	0·0467		
Wheat embryo	0·1	4	2	0·75	−3·0	−1·12	0·0101	1 : 3·5	1 : 3
	0·3	2	4	39·5	27·25	33·37	0·0352		
Yeast extract I	0·05	3	2	−6·7	−2·5	−4·6	0·0090	1 : 3·8	1 : 4
	0·2	3	2	39·3	33·0	36·15	0·0341		

response constructed about the same time and with the use of the same basal diet (containing 8 % autoclaved yeast). When these are interpreted by means of the second curve of response (basal diet containing 20 % autoclaved yeast), the ratios of the apparent vitamin B_1 potencies of the respective pairs of doses are found to be in very good agreement with the known ratios of the doses given.

This may be taken as good evidence that the basal diet which contained only ·8 % autoclaved yeast was deficient in some factor necessary for growth which was not supplied by the activated acid clay but which was supplied by each of the other substances examined.

The potency of all substances examined has, therefore, been calculated by means of the second curve and stated in terms of the I.S. for vitamin B_1 in Table VIII.

Table VIII. *Potency of substances tested by the rat method in terms of the I.S. for vitamin B_1.*

Substance tested	Dose (g.)	Abscissa corresponding to mean increase in weight	No. of units vitamin B_1 per g.	
Dried yeast I	0·05	0·0096	19·2	Average 19·8
	0·2	0·0407	20·35	
Food substance	1·0	0·0226	2·26	" 1·9
	3·0	0·0467	1·56	
Wheat embryo	0·1	0·0101	10·01	" 10·9
	0·3	0·0352	11·73	
Yeast extract I	0·05	0·0090	18·0	" 17·6
	0·2	0·0341	17·05	
Dried yeast II	0·05	0·0125	25·0	" 24·7
	0·15	0·0365	24·4	
Yeast extract II	0·01	0·0160	16·0	" 14·4
	0·2	0·0255	12·8	

Discussion.

The point that appears to us of the greatest interest in this examination of the growth response of rats to graded doses of vitamin B_1 is that the slopes of the two curves of response obtained with 8 and 20 % respectively of autoclaved yeast in the basal diet are different. One might have expected the two curves to be superposed at their lower ends and the curve obtained with 8 % autoclaved yeast to bend away suddenly from the other one at the point where the maximum effect allowed by 8 % autoclaved yeast was reached. This, however, is not what happens. There is no sudden limiting of the response to vitamin B_1 when the supply of vitamin B_2 is insufficient. The inadequacy of the supply of vitamin B_2 affects the response to lower doses of vitamin B_1 as well as to higher doses. Thus, with only 8 % autoclaved yeast in the basal diet, a dose of some substance containing vitamin B_2 as well as vitamin B_1 would give a higher result than the same amount of vitamin B_1 in a substance free from vitamin B_2; provided the results fell on the curve, no error would be suspected (*cf.* our results depicted in Fig. 4) and the estimation of vitamin B_1 would be too high. This is not only of practical importance, but it seems to be of theoretical importance also as indicating an interdependence of the vitamins.

The difference in slope of the curves seems to indicate that the more vitamin B_2 (or some other factor, not B_2 or B_4, in the autoclaved yeast) in the basal diet, the greater is the response of rats to doses of vitamin B_1. Below the point of intersection (5 g. mean increase, 0·0125 g. Standard), the reverse appears to be true.

This is a similar result to the one reported by us [Coward *et al.*, 1932] concerning the dependence of the response to vitamin D on the amount of vitamin A in the basal diet (or given daily). We found that the larger the dose of carotene given to all rats daily, the greater was the slope of the curve of response to graded doses of vitamin D.

We do not conclude from these experiments that 20 % yeast autoclaved at 120° for 5 hours will always provide sufficient of the B vitamins (other than B_1)

to support normal growth. Indeed we have evidence (to be reported in a later paper) that 20 % of yeast autoclaved apparently in the same way may be far too small an amount for normal growth. We consider that our results demonstrate afresh the need for making a simultaneous test on at least one dose and preferably on two doses of a standard of reference whenever an examination of the vitamin B_1 content of a substance is made. The test can only be considered satisfactory if there is no evidence (such as we described in Section III) that the substance under test is supplying some factor other than vitamin B_1 which is not contained in sufficient amount in the basal diet.

PART III. THE VITAMIN B_1 POTENCY OF A SUBSTANCE AS ESTIMATED BY MEANS OF (*a*) PIGEONS AND (*b*) RATS.

The decision whether the tests by means of pigeons and the tests by means of rats measure the same factor rests on the results obtained from the examination of substances by both the methods. If both methods give the same result it may be assumed that the two methods measure the same factor. Two substances have been examined by both methods: (*a*) a soft extract of yeast I and (*b*) a sample of dried yeast I (Table IX).

Table IX.

	Potency of substances as determined by tests on		
	(*a*) pigeons		(*b*) rats
Substance tested	as calculated by day-dose method (units)	as calculated from % birds cured (units)	as calculated from mean increase in weight in 3 weeks (units)
Soft extract of yeast I	20·5	18·5	17·6
Dried yeast I	42·0	34·0	19·8
Dried yeast III	113·0	83·0	37·8

There is good agreement between the results of the tests of the soft yeast extract obtained by all three methods, but the pigeon test of the dried yeast makes it appear to be twice as potent as does the rat test. The chance that this divergence is due to the inaccuracy which is inevitable in all three tests has been calculated as about 1 in 4000, which is exceedingly small. It appears therefore, that for dried yeast, pigeons give a higher value for the vitamin B_1 potency than do rats. Confirmation of this result has recently been obtained by the examination of a third sample of dried yeast (III, Table IX)[1]. The day-dose method gives results which vary according to the size of dose given as shown in Parts I and IV of this paper, therefore very little attention need be given to the result obtained by this method of calculating the potency of the dried yeast. The result from the percentage of birds cured, however, confirms the result obtained with the first sample of dried yeast; *viz.* the pigeon method of calculating the vitamin B_1 potency of dried yeast gives a higher figure than the rat method. Actually the potency obtained by the pigeon method is about double of that obtained by the rat method.

[1] This test was made in the course of a comparison of different workers' methods of estimation of vitamin B_1 arranged by Prof. A. Jung, University of Basel.

It appears, therefore, that there is some reason for thinking that the rat is less able to respond to the vitamin B_1 contained in dried yeast than is the pigeon. On the other hand, the rat gave the same result as did the pigeon for the soft extract of yeast I, and from this it must be concluded that the rat and pigeon can respond equally well to the vitamin B_1 in such an extract and also to that in the acid clay. Thus the rat-growth method may prove to be unsuitable for estimating the B_1 potency of some substances, but, on the other hand, polyneuritic pigeons are obviously not suitable for estimating the vitamin B_1 potency of bulky substances such as wheat germ or wholemeal bread. The pigeon test moreover has a probable error of +31 or −26 % when 9 birds are used for a single dose of a substance, whereas the rat test has a probable error of only +6·1 to −5·5 % when 9 animals are used. It must be recognised, however, that the pigeon test is one in which the estimation is based on a specific reaction to vitamin B_1 and it must, therefore, on general principles, be preferable to a growth test.

PART IV. FURTHER CONCLUSIONS DRAWN FROM A STATISTICAL EXAMINATION OF THE RESULTS.

I. Pigeons.

A. Method of calculating results by duration of cure.

(a) Standard deviation of the average duration of cure.

The mean variance of the number of days of the pigeons' cure was calculated from the 19 tests recorded in Table II by the formula $\sigma^2 = \frac{\Sigma d^2}{N - m}$ where N = the number of observations (*i.e.* the number of pigeons) and m = the number of means (*i.e.* the number of tests). It was found that

$$\sigma^2 = 9{\cdot}02,$$

whence $$\sigma = 3.$$

The number of pigeons from which the calculation was made is admittedly small, but it is large enough to use the value of σ found as a basis for certain calculations.

(1) *The standard deviation of the duration of the cure in relation to the duration of the cure.* It was to be doubted whether the variation in length of cure would be the same for pigeons given small doses as for pigeons given large doses of vitamin B_1. The results were, therefore, divided into 3 groups according to the average number of days of cure of the pigeons of the groups. The mean variance for each group was calculated and from it the standard deviation (Table X).

Table X.

No. of pigeons in group	Average no. of days cured	Standard deviation of mean
46	2·0	1·2
54	4·1	3·03
60	6·4	3·75

Thus there seems to be some evidence that there is less variation in the results obtained with low doses of vitamin B_1 than in those obtained with high doses.

(2) *Comparison between solids and liquids with regard to the pigeon's power of extracting and absorbing vitamin B_1 from them.* It might be expected that a pigeon in an abnormal state of health would be less able to utilise the vitamin B_1 in a solid preparation such as dried yeast or even activated acid clay than that in a liquid preparation such as Peters's concentrate, the liquid extract of rice polishings or the soft extract of yeast which was diluted with water before being given to the bird. Calculations were, therefore, made of the mean variances found in the tests on the liquid and solid preparations respectively. The values of σ ($= \sqrt{\text{mean variance}}$) of the two groups were found to be 2·93 and 3·06. These cannot be regarded as different. As the average duration of a cure of all the pigeons given a liquid preparation was 3·96 and that of all the pigeons given a solid preparation was 4·60 days it is evident that the similarity in the values of σ for the two groups has not arisen through a difference in absorbability being balanced by a difference in the average duration of cures of the two groups. Thus it must be concluded that pigeons such as were used in these tests can absorb vitamin B_1 from liquids and from solids such as the I.S. and dried yeast equally well.

(*b*) *Relation of duration of cure to dose of vitamin B_1 given.*

A curve has been constructed relating the duration of cure to dose of vitamin B_1 given. The data obtained from the doses of acid clay alone were not considered sufficient for constructing a curve for general application. Therefore, the acid clay equivalent of each dose of substance tested was calculated from the average value of that substance stated in Table III. The results were then averaged in 6 groups according as the dose given was (*a*) 0·011 to 0·016, (*b*) 0·021 to 0·024, (*c*) 0·030 to 0·032, (*d*) 0·040 to 0·048, (*e*) 0·056 to 0·060, (*f*) 0·085 to 0·087, (*g*) 0·12 to 0·175, (*h*) 0·35 g. of acid clay or its equivalent, and plotted as a curve relating average duration of cure to dose of acid clay (I.S. for vitamin B_1) (Fig. 7, continuous line). This curve is not logarithmic.

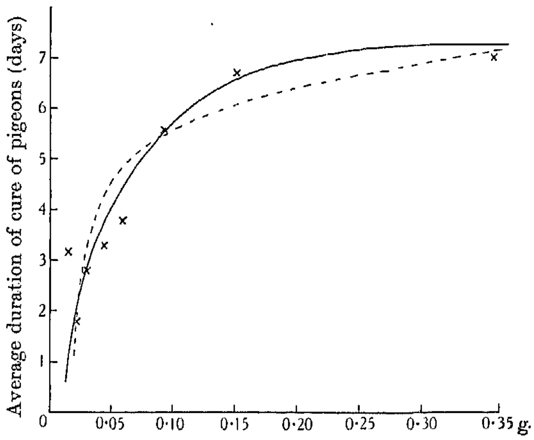

Fig. 7. Curve of response constructed from the duration of cures of polyneuritic pigeons given graded doses of activated acid clay or the equivalent of these doses in other substances.
Continuous line—best curve drawn through points obtained experimentally.
Broken line—best logarithmic curve through these points, but not adopted as it fits the points much less well than the other curve.

The best logarithmic curve through the points has been drawn as a broken line. It does not fit the points as well as the first curve drawn and there seems to be no reason for expecting the curve to be logarithmic; it has not been used.

(c) *The degree of accuracy obtainable by this method.*

The probable error of this test as obtained under the conditions described for this laboratory has been calculated in Table XI.

Table XI. *The probabilities of obtaining certain degrees of accuracy in this test. Standard error of duration of cure when 9 birds are used in a test.*

$$\epsilon = \frac{\sigma}{\sqrt{n}} = \frac{3}{\sqrt{9}} = 1.$$

Average duration of cure, say,	Abscissa corresponding to duration of cure		Range of duration of cure	Range of abscissae* corresponding to range of cure	% range of abscissae
4 days	0·049	(*a*)	$\pm\frac{2}{3}\epsilon = \pm 0{\cdot}67$ ($\frac{1}{2}$ chance)		
			4·67	0·064	130·6
			3·33	0·036	73·5
		(*b*)	$\pm\epsilon = \pm 1{\cdot}0$ ($\frac{2}{3}$ chance)		
			5·0	0·074	151·0
			3·0	0·032	65·3
		(*c*)	$\pm 2\epsilon = \pm 2{\cdot}0$ ($\frac{21}{22}$ chance)		
			6·0	0·110	224·5
			2·0	0·022	44·9

* Obtained by direct reading from curve (continuous line).

Thus the probable error of an estimation of vitamin B_1 by the use of a curve relating duration of cure to dose of vitamin B_1 given and using 9 pigeons for one dose of the substance under examination is about +31 or −26 %. We have not yet sufficient evidence to say whether a simultaneous test on the Standard should be made with every test of an unknown substance. On general principles it would seem to be desirable. The probable error of the estimation of the unknown in terms of the Standard then becomes $(+31 \text{ or } -26) \times \sqrt{2}$ which becomes +43·7 or −37·0 %.

It is obvious from the shape of this curve of response that, as pointed out in the early part of this paper, if a high dose of vitamin B_1 is given the result calculated as the day-dose response (*i.e.* days of cure/dose) will be lower than when a low dose of vitamin B_1 is given. The method of calculating the potency of a substance from days of cure/dose assumes a straight line relationship between duration of cure and dose given. The work in this paper shows that the relationship is curvilinear. Therefore, the day-dose method of calculating results can only be used over a very limited range of duration of cure. It resolves itself into a determination of the dose of the unknown substance required to give a cure of some chosen number of days and leaves no means of interpreting results which are of longer or shorter duration than the period chosen.

(d) *Confirmation of the curve relating duration of cure to dose of vitamin B_1 given.*

In the fifth column of Table XII are given the doses of acid clay corresponding to the mean duration of cure in each test. In the last column is stated in units per g. the potency of each substance as determined from each dose tested. The results from the different doses of any one substance are in good agreement

Table XII. *Vitamin B_1 potency of different substances in terms of the I.S., estimated by duration of cure method.*

Material	Dose	No. of birds cured	Average duration of cure (days)	Equivalent dose of acid clay (g.)	No. of units per g.
Acid clay (original sample)	0·015 g.	4	3·75	0·0435	290
	0·03 g.	11	3·7	0·0425	142
	0·06 g.	16	4·4	0·058	97
	0·12 g.	13	7·7	0·320	267
Acid clay (Standard)	0·03 g.	7	2·1	0·022	73
Dried yeast	0·031 g.	2	4·5	0·0605	195
	0·062 g.	5	1·8	0·020	32
	0·125 g.	15	4·4	0·058	43
	0·25 g.	16	5·9	0·1055	42
Vitamin B_1 solution (*a*)	0·1 cc.	4	2·0	0·021	21
(*b*)	0·2 cc.	6	1·8	0·020	10
(*a*)	0·25 cc.	7	2·1	0·022	9
(*b*)	0·35 cc.	9	2·5	0·027	8
Extract of rice polishings	0·25 cc.	5	1·6	0·0185	7
	0·5 cc.	10	5·1	0·0775	15
	1·0 cc.	10	5·7	0·098	10
	2·0 cc.	11	7·5	0·256	13
Yeast (soft extract I)	0·12 g.	3	1·7	0·019	16
	0·24 g.	6	3·5	0·039	16

except for (*a*) certain doses for which a relatively small number of animals was used, and (*b*) the highest dose of acid clay of which the result has to be interpreted from a very high, and therefore unreliable, part of the curve. The curve may, therefore, be considered a fairly reliable one for relating duration of cure to dose of vitamin B_1 given.

Moreover this curve and the curve relating percentage of birds to dose of vitamin B_1 give the same results within the limits of accuracy of the tests.

B. Method of calculating results by percentage of birds cured.

(*a*) *Standard deviation of the percentage number of birds cured.*

The percentage standard error of each of the 19 tests was calculated from the formula $\epsilon = \sqrt{\frac{p.q}{n}}$ where p = the percentage of pigeons cured, q = the percentage not cured, n = the total number of pigeons used in the test. From each value of ϵ the corresponding value of σ was calculated ($\sigma = \epsilon\sqrt{n}$). The weighted mean of the 19 values for σ was found to be 43·08 %.

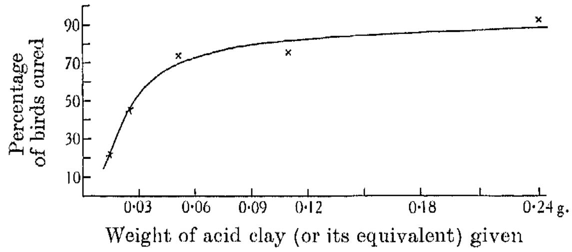

Fig. 8. Curve of response constructed from percentage of polyneuritic birds cured by doses of activated acid clay or the equivalent of these doses in other substances.

(b) *Relation of percentage of birds cured to dose of vitamin B_1 given.*

A curve of response (Fig. 8) has been constructed by grouping the pigeons and averaging results as in the last section. This curve is, therefore, based on more data than that in Fig. 2, it is extended through higher doses and it relates percentage of birds cured directly to the corresponding dose of the standard.

(c) *The degree of accuracy obtainable by this method.*

The degree of accuracy of an estimation by the use of 9 pigeons has been worked out as for the calculation of the duration of cure. It is summarised in Table XIII.

Table XIII. *The probabilities of obtaining certain degrees of accuracy in this test. Standard error of percentage of birds cured when 9 birds are used in a test.*

$$\epsilon = \frac{\sigma}{\sqrt{n}} = \frac{43{\cdot}08}{\sqrt{9}} = 14{\cdot}36.$$

Percentage no. of pigeons cured, say,	Abscissa corresponding to % cured	Range of percentage number cured	Range of abscissae* corresponding to range of % number cured	Percentage range of abscissae
50	0·028	(a) $\pm\frac{2}{3}\epsilon = \pm 9{\cdot}57$ ($\frac{1}{2}$ chance)		
		59·57	0·036	129
		40·43	0·021	77
		(b) $\pm\epsilon = \pm 14{\cdot}36$ ($\frac{2}{3}$ chance)		
		64·36	0·042	150
		35·64	0·019	67
		(c) $\pm 2\epsilon = \pm 28{\cdot}72$ ($\frac{21}{22}$ chance)		
		78·72	0·084	300
		21·28	0·014	50

* Obtained by direct reading from curve of response.

It is evident from the figures in the last column of Table XIII that the curve relating the percentage of birds cured to dose of vitamin B_1 given is somewhat flatter at its upper end than the curve relating the duration of cure to dose of vitamin B_1 given. Except for results falling on the upper part of the curve, which one would try to avoid in either test, it is evident that equal degrees of accuracy are obtained in the estimation of vitamin B_1 by the use of polyneuritic pigeons in either way, provided the results are interpreted by means of curves of response such as we have constructed for this paper.

2. Rats.

The growth response.

(a) *The standard deviation of the response to a dose of vitamin B_1.*

The mean variance of the increase in weight in 3 weeks of rats given a dose of vitamin B_1 after they have ceased to grow on a diet deficient in this vitamin is 89·06 for bucks and 38·96 for does. Thus the standard deviation of the increase in weight in 3 weeks is 9·4 for bucks and 6·2 for does. These figures are presented with some hesitation as they were calculated from only 47 bucks and 36 does respectively. For purposes of calculating the degree of accuracy obtainable in the estimation of vitamin B_1 as carried out by the growth-promoting test in this laboratory, the two values for σ have been averaged ($M = 7{\cdot}8$).

(*b*) *The degree of accuracy obtainable by this test.*

If 7·8 be accepted as the value for σ then the standard error of the test using 9 rats on a dose is $\epsilon = \frac{\sigma}{\sqrt{n}} = \frac{7\cdot 8}{3} = 2\cdot 6$. The degree of accuracy may then be calculated from the equation of the curve of response, $y = 139\cdot 4 + 70\cdot 4 \log x$ (Table XIV).

Table XIV. *Degree of accuracy obtainable with 9 rats on a dose.*

$$\left[\epsilon = \frac{\sigma}{\sqrt{n}} = \frac{7\cdot 8}{3} = 2\cdot 6\right].$$

Average increase in wt. in 3 weeks, say,	Abscissa corresponding to this increase	Range of increase in wt. in 3 weeks (g.)	Range of abscissae corresponding to range in increase in weight	% range of abscissae (doses)
20 g.	0·0201	(*a*) $\frac{2}{3}\epsilon = \pm 1\cdot 74$ ($\frac{1}{2}$ chance)		
		21·74	0·0213	106·1
		18·26	0·0190	94·5
		(*b*) $\epsilon = \pm 2\cdot 6$ ($\frac{2}{3}$ chance)		
		22·6	0·0219	108·6
		17·4	0·0186	92·6
		(*c*) $2\epsilon = \pm 5\cdot 2$ ($\frac{21}{22}$ chance)		
		25·2	0·0239	118·9
		14·8	0·0170	84·5

Similarly the percentage range of abscissae, *i.e.* the percentage range of accuracy obtainable when only 4 rats are used on a dose of vitamin B_1 is:

1 in 2 chance (probable error)	92·6 to 108·6 %
2 in 3 chance	88·2 to 113·8 %
21 in 22 chance	77·6 to 129·5 %

Thus the degree of accuracy obtainable in an estimation of vitamin B_1 appears to be very high. As, however, the value for the standard deviation is based on only a small number of animals, it is possible that the degree of accuracy may not be as great as this, though it is unlikely that it will be very much less.

Summary.

New methods are proposed for estimating the potency of preparations containing vitamin B_1 by means of (1) tests in which pigeons are cured of head retraction, (2) tests in which the growth of rats is observed. Several substances have been examined by these methods in comparison with the International Standard (I.S.).

The responses of pigeons (duration of cure and percentage of birds cured) and of rats (growth) to doses of vitamin B_1 are curvilinear. This fact must produce differences in results calculated by Peters's day-dose method according as the dose tested is high or low. With pigeons, the duration of cure and the percentage of birds cured give the same estimation of potency provided the results are interpreted by means of suitable curves relating response to dose of vitamin B_1 given.

The rat and pigeon methods give the same estimation of potency of a soft extract of yeast in comparison with the I.S. Thus the rat and pigeon appear to have equal ability for dealing with the vitamin B_1 in the Standard. In tests

of two different samples of dried yeast, however, the pigeon test indicated a vitamin B_1 potency about double that indicated by the rat test. Thus the rat appears to be less able to respond to the vitamin B_1 of dried yeast than does the pigeon. Statistical estimations of the results indicated that the probable errors of the tests using 9 animals in a test are:

(1) pigeon, (*a*) duration of cure ... ... $+31$ or -26 %,
(*b*) percentage cured ... ... $+29$ or -23 %;

(2) rat, mean increase in weight in 3 weeks ... $+ 6{\cdot}1$ or $- 5{\cdot}5$ %.

Moreover, the standard deviation of results obtained by pigeons is no greater in tests on solids (*e.g.* acid clay or dried yeast) than it is for liquids (*e.g.* extracts of yeast). Therefore, there is apparently no greater variation in the ability of different birds to deal with solids than in their ability to deal with liquids.

In spite of the probable error of the pigeon test being much greater than that of the rat test, the former has the great advantage of being specific for the factor it is used to estimate. It may also be that it gives a truer result with certain substances (*e.g.* dried yeast) than the rat test, though it may be difficult to use the polyneuritic pigeon for testing many substances which cannot be suspended in water and given by a stomach tube.

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