XXXVIII. ANTINEURITIC YEAST CONCENTRATES. III.

THE CURATIVE PIGEON TEST: A CRITIQUE.

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PART I.

Standardisation of the test.

Curative tests upon the pigeon have two main advantages over other methods of testing for the antineuritic principle. Firstly, it is not yet absolutely certain that rat tests can differentiate the curative factor in the vitamin B complex. This matter has been discussed elsewhere [Kinnersley and Peters, 1927], so that it is not necessary to reiterate the evidence here. Secondly, the pigeon is an easy bird to obtain, and to manage, and further it gives a rapid answer with small amounts of test material. It has been the object of the tests carried out in this Department during the last four years to discover the errors to which the test is liable and to learn how to avoid them. An account of the position of the pigeon test is needed at the present time, owing to the appearance of statements in the recent literature tending to discredit the use of the pigeon for short curative experiments. Jansen and Donath [1927] for instance discarded the pigeon as a test object owing to confirmation of Theiler's experiment, in which it was found that birds could be occasionally cured by injections of distilled water. Eijkman [1927] lays great stress upon the necessity of prolonged tests upon the pigeon. In a recent paper by Plimmer, Rosedale, Raymond and Lowndes [1927] the authors have used both curative and preventive tests. They have found that the results obtained with the curative test depend upon the state of the bird, and that, though there is general agreement between the preventive and curative tests, occasionally there are wide divergences. These discrepancies may be due to some inherent error in the curative tests; they may however be ascribed to the fact that preventive tests carried out over prolonged periods such as 60–100 days test for more than one factor. This latter explanation would seem to be the conclusion to be drawn from the results recorded in this communication.

In an earlier paper by one of us [Peters, 1924], certain conditions were enumerated as essential for the success of the curative tests. These were briefly as follows. Only the birds which show the classical symptoms of head retraction should be used. Of these only those showing the symptoms before 30 days can be relied upon, and then only if they seem to be strong enough to stand the test. It was further emphasised that unless a cure was effective for more than 24 hours, it must be ascribed to some temporary effect. An example of such a temporary cure was described, in which the administration of distilled water (by the mouth) cured for a few hours. The test as described in the early paper was used in working out a method of preparing concentrates from yeast of an activity of about 3 mg. per day. In spite of the successful use of the test in preparing these concentrates, other results appeared of a puzzling nature. It was found for instance that small doses of histamine would often produce genuine cures (5 out of 18 birds treated). That such an effect was not due to the presence of minute traces of adsorbed vitamin in the histamine samples was shown by proving that the onset of symptoms was not affected by giving daily doses of histamine. Thus histamine could not function as a protective substance though it could occasionally cure. This confirmed certain earlier conclusions of Abderhalden [1923, 1]. Among a number of other substances tried, a cure was produced by olive oil. Other points brought forward in the same paper were the importance of avoiding cold shock, and the value of keeping the pigeons in the early stages of feeding in a relatively cold place. In a subsequent paper [Kinnersley and Peters, 1925] the technique was slightly modified. The birds were kept in a cool room until the symptoms had either begun to develop or were threatening. They were subsequently transferred as soon as possible to a warm room, and left there for a matter of 2 hours at least before commencing the tests. It was found that such birds would often clear up for 1–3 days, the condition being known as "heat cure." This technique has not been varied significantly since then except in regard to the administration of glucose, to which reference will be made below.

The curative test has been used quantitatively by noting the number of days after cure for which the bird is protected from a second appearance of symptoms. It has been tacitly assumed that only one factor is concerned in producing the cure and the subsequent protection. This is only likely to be true where comparatively short periods of experiment are concerned. As a general rule, the test has been sufficiently quantitative to cause no difficulty in following the distribution of activity during a fractionation. However, as experience of the birds has increased, it has become clear that there are always some abnormal cases. Occasionally this abnormality has become sufficiently serious to disturb the tests. One such abnormal case was shown in Table I [Kinnersley and Peters, 1925]. Whereas the average birds reacted to doses of the preparation of between 0·068 and 0·11 mg., the abnormal case only required 0·025 mg. of the preparation *per diem*. These cases therefore

require much less than an average dose to cure and protect from a further onset of symptoms. In view of this type of abnormality, it is important to enquire whether the quantitative nature of the test is a mere illusion. Especially pertinent is this question in view of the cases of so-called spontaneous cure described by Kon and Drummond [1927], though these have been shown by Kon [1927] not to occur upon rice diets. If the question is considered theoretically, it might be conceived that any process which leads to the cure of a bird will free that bird from further symptoms for an arbitrary period of 2–7 days. In this case the number of days of protection would have only a pseudoquantitative nature, and would be actually due to some random selection and averaging of favourable cases. It is believed by the authors that such a view is incorrect, though it is difficult to obtain a rigid proof of the question owing to the accidental happenings to which the test is liable (such as unobserved vomiting of the dose upon the part of the bird). Three classes of evidence may be adduced in favour of a direct quantitative relation between the dose and the time of protection after cure.

(1) The test has enabled us to work out methods of concentrating the factor some 20,000 times, as compared with pressed baker's yeast (1·0 g. to < 0·05 mg. dry dose).

(2) The administration of a second test dose following upon onset of symptoms after a first dose reproduces the results of the first dose.

(3) The distribution of the curative substance can often be followed quantitatively in a fractionation.

(1) needs no further comment.

(2) In Table I there has been collected a number of instances in which it has been possible to administer a second dose of the same extract as was used for the first dose immediately upon a second appearance of symptoms. Unless such a dose is given soon after the reappearance of symptoms, no conclusions can properly be drawn from the results owing to the ease with which the bird passes beyond recovery.

Table I. *Successive dosing.*

(A) Cases in which the second dose was the same in amount as the first.

Bird	Protection for: First period	Second period
428	5 days	5 days
649	3 ,,	3 ,,
544	3 ,,	3 ,,
549	5 ,,	3+ ,,
790	3 ,,	5 ,,
709	3 ,,	4 ,,
587	3 ,,	2 ,,
713	3 ,,	4–5 ,,

(B) Cases in which the second dose was double in amount.

581	2 days	4 days
908	3 ,,	6 ,,

N.B. + means that the bird was cleared up with marmite before head retraction had occurred again.

In each of these cases, except No. 649, the symptoms were those of head retraction. In No. 649 they were the so-called forward symptoms (McCarrison's emprosthotonus). In cases (B) twice the dose was given upon the second occasion. These experiments suggest that an individual bird reacts in a standard way to a given preparation. The (B) cases are especially interesting in so far as they show that the number of days' cure for a given bird can be varied by varying the dose. The results of Table I are difficult to explain upon a theory of random sampling.

(3) Many examples have been obtained in the course of the work in which, during a fractionation, the final doses obtained from precipitates and filtrates have added up to a value very close to the original number. Space does not admit the giving of these in detail, but two instances may be quoted. In a silver fractionation upon material of original activity 1·2 mg., 425 doses could be accounted for out of an original 450. Again in the course of a charcoal fractionation 710 doses were recovered out of 720 in the original. These favourable results are not always obtained, a matter which will be discussed below, but the fact that they can be produced upon occasion suggests strongly the underlying quantitative character of the response.

Taken collectively, the evidence above is enough to show that pigeons test for some definite substance in a quantitative manner.

Certain difficulties. Accepting the view however that there is fundamentally a relation between the amount of the factor supplied and the time of protection after cure, there is no doubt that a proportion of the birds behave abnormally. Excluding cases due to obvious experimental error (such as improper administration of the dose), some birds in each batch and occasionally many in a given batch behave irregularly in the sense that much less of a given preparation is needed to cure and protect them than the average. It is clear that unless such cases can be reduced to a minimum or controlled, the test becomes unreliable. We have attempted to explain these abnormalities. At first they were attributed to faulty technique, as it is noticeable that at these times a smaller proportion of the birds develop typical symptoms before the 30th day. Improper cleaning of the cages and consequent feeding upon excreta might lead to this result. However, special attention to these details did not improve the position. One period when it was especially evident was in November and December 1926, when the matter was sufficiently serious to upset the tests. An illustration will perhaps make clearer the nature of the difficulty. During the course of an alcohol fractionation, the results shown in Table II were obtained.

There is a wide variation during the fractionation, from 600–1870 plus. "Plus" represents the fact that the bird was cleared upon the 10th or 11th day by marmite before it had shown symptoms again. After 10 days results are not reliable. It might at first be thought that these results could be explained by a difference in the weight of the birds. Reference to Table II will show that this is not so. It might be thought also that a bird which

showed symptoms earlier needed more of the active principle than one maturing later upon a rice diet. With the possible exception of 599, this does not seem to be true.

Table II. *Alcohol fractionation. Case of bad variation in tests.*

During the successive removal of precipitates, the following bird results were obtained. (*a*), (*b*), etc. means result obtained at successive stages.

Stage	Bird	Result in day doses	Weight of bird at onset of symptoms	Day of symptoms
(*a*)	286	750	285 g.	25th
(*b*)	532	1700 +	205	24th
(*c*)	501	1870 +	300	28th
	599	600	285	10th
(*d*)	606	1000 +	235	24th

Upon a reference to the books it became clear that the birds which gave such a large proportion of abnormal results came from a batch which had been placed upon the polished rice diet immediately upon receipt from the dealers. Out of a batch of 30 birds, only 12 were obtained with the correct symptoms for treatment, and of these 12 at least 8 gave abnormally high answers. This suggested that some storage owing to a previous period of feeding was influencing the results. Such storage effects are already very familiar to workers upon the fat-soluble vitamin. The matter was tested directly by comparing the behaviour of two batches of birds under exactly comparable conditions, as regards temperature, feeding and exercise. One batch of 8 birds (A) was placed upon the polished rice diet upon receipt from the dealer, the other (B) of 11 birds was standardised before polished rice feeding upon a mixed diet in the laboratory for a month. A summary of the results obtained is given in Table III.

Table III.

(A) 8 birds. Placed on polished rice diet immediately upon receipt from the dealer.

Bird No.	Colour	Weight when symptoms appeared g.	Day of symptoms	Days' cure and protection
787	Grizzle	215	16th	8
790	Blue bar	245	23rd	3, 5*
789	,,	260	27th	5
704	Grizzle	230	29th	5
[794	Blue chequer	260	31st	(12)]

No symptoms were shown by the 38th day by three other birds, one of which was blue and the others red.

(B) 11 birds. Placed on polished rice diet after previous period of laboratory feeding.

428	Blue bar	310	16th	2
722	White	235	16th	5
709	Mealy	225	20th	3, 4*
549	Blue chequer	275	21st	7
684	Blue chequer pied	285	21st	4
?	Grizzle	—	23rd	No test
587	Blue chequer	285	23rd	3, 2*
649	Mealy	230	27th	2
713	White	230	28th	3, 4½*

No symptoms shown by two other birds by 38th day. One of these was a blue bar, and the other a grizzle.

* Indicates that a second dose following the first gave the second figure.

As each case developed, the bird was given a dose of 0·2 cc. of a standard curative preparation. Comparing the behaviour of the two batches, a larger proportion of those in Batch B showed symptoms before the 30th day, 81 % in B against 63 % in A. Of the birds received fresh from the dealers 3 never showed symptoms at all. Also out of the 5 cases from Batch A, 2 gave abnormally high responses, namely, 787 and 794. It is not a question of the day upon which symptoms appeared, because one showed these upon the 15th day whereas the other did not show anything until the 31st day. Of the cases obtained from Batch B, only one case, namely 549, gave an unreasonable response as compared with the others. This proves that the previous feeding influences the effects produced by a period of rice feeding. Further conclusions may be drawn from these results. There is no correlation between the colour of the bird and behaviour to the diet or to the test dose. This reversed a previous impression which we had obtained that the white bird was more sensitive. Also there seems to be no correlation between time of onset of symptoms, length of protection after cure and weight of birds at the onset of symptoms.

If we confine ourselves to Batch B, it will be seen that 9 of the tests gave responses between 2 and 4 days, and that 2 others fell outside these limits at 5 and 7 days.

Taken apart from the evidence in the earlier part of the paper, these results might be construed to mean that any agent which cures, does so for a period merely depending upon the bird and that there was no true quantitative happening. If we confine ourselves to the results of Batch B alone, there seems to be a better case for quantity. In one respect these results were not satisfactory. The stock solution used for dosage was found subsequently to contain rather large amounts of metals, which we have found to be apt to cause irregular results. The matter was left until the summer vacation when the opportunity offered for reinvestigation. It was decided to test again the influence of standard feeding upon the number of cases obtained in the 30-day period; and further to see whether, with standardised birds, more standard responses to a given test dose could be obtained with the same preparation as that used in the experiments of Table III, but after it had been freed from metals. The stock birds were therefore fed for 5 weeks upon the mixed laboratory diet, and subsequently placed upon polished rice. The results were rather surprising. Practically all birds placed upon the polished rice developed the characteristic symptoms before the 30th day, in some batches of 20 every bird became a case for experiment. This proved that birds before test should be fed in a standard way.

Even among these birds the abnormal case still appeared, though less frequently. Before continuing with the description of the further experiments with the stock preparation, it is necessary to describe a further improvement in technique, which has been introduced with a view to eliminating the abnormal case. In the hope of explaining some of the abnormal responses

obtained, especially with preparations of under 0·1 mg. per day activity, small doses of glucose were given with some of the preparations. This led to a test of the effect of small doses of glucose (20–50 mg.) given alone. To our surprise these doses seemed to be capable of curing certain birds. The glucose sample had been prepared by one of us (V. R.) by purification with charcoal and subsequent recrystallisation from 75 % alcohol. It contained no trace of the *Streptothrix* growth factor[1], and it is inconceivable that the effect could be due to adherent vitamin. An amount of 50 mg. may seem to be too small to have food value, until it is remembered that the weight of these birds is not more than 250 g. If the weight of the blood be placed as high as one-twelfth of the body weight and the blood-sugar at 0·2 %, then the amount of sugar in the blood of one of these pigeons would be 40 mg. In most cases, like the "heat" cures, the "glucose" cures do not persist for long, and cannot be repeated by giving a further dose. In two cases it has been found that doses of glucose would cure a bird even for a second time. Table IV gives the details of these two cases.

Table IV. *Glucose.*

Case A. Bird 981. Blue chequer pied. Developed symptoms upon 23rd day of feeding, and was given 20 mg. glucose. Bird cleared up for 7 days and was then given a further 20 mg. glucose which again cleared it up for two more days. Upon the third occasion glucose had no effect and the bird died.

Case B. Bird 60. Blue chequer. Brought into warm room with threatening convulsions upon 22nd day of feeding. Upon 23rd had head retraction, and was given 20 mg. glucose which cured in 4 hours. The cure lasted until 25th day, when more glucose was given. This cured the bird until the 26th day, when it was used for experiment. In this case it is certain that temperature played no part in the results.

It is proposed to return to a discussion of the "glucose" cure later. For the purpose of the further experiments upon the stock preparation it is sufficient to note that the further precaution was taken of giving all the test birds a dose of purified glucose as well as allowing ample time for the development of any possible heat cure, before giving the test dose.

Table V.

Preparation. Stock H. 0·1 cc. = 1/1900 of the whole.

Dose	Bird	Day on which symptoms appeared	Days of cure and protection
0·1 cc.	37	23rd	2
	52	24th	2
	[60	24th	(6)?] abnormal
	2	31st	2
0·2 cc.	30	—	3
	1	23rd	4
	56	30th	4
	98	18th	7
	66	28th	7

Table V gives the results of tests carried out upon birds standardised by a period of feeding for at least a month upon the mixed laboratory diet. The questions to be answered were; firstly, whether birds selected at random for test behaved in a standard way to a given test dose; secondly, what was

[1] This work is being prepared for publication.

likely to be the limits of variation in the number of days of protection after cure for a given dose; and, thirdly, whether increase of the dose increased the number of days of protection after cure proportionately. It had been our impression that a small dose upon the margin of the curative one (2 days and under) gave relatively a low result as compared with a higher dose.

The results are in general less variable than those of Table III. The abnormal case has been included in the table because of its interest. It stamped itself as abnormal by the fact that the bird was twice cleared up by doses of glucose. Excluding this case, which would be automatically excluded by the present methods of testing, the results are, for a dose of 0·1 cc. 2 days, for 0·2 cc. 5 days $\pm$ 2. These seem to be the best results which we can obtain for pigeons selected at random for the tests. For larger doses see Kinnersley and Peters [1925, p. 821]. In answer to the third question, it is seen that increase of the dose has increased the number of days of protection after cure, though not quite proportionally. If we make the assumption, which is reasonable, that one dose is used in clearing the bird and add one day to each of these figures, there is exact correspondence between the figures, namely, 0·1 cc. 3 days, 0·2 cc. 6 days. The variations of the test from the mean seem to be $\pm$ 40 %. By taking the average of two birds, the chances of wide variation are of course much reduced.

The above experiments appear to establish the fact that there is a quantitative relation between the time of protection after cure and the amount of the curative dose given, provided that short periods of protection are considered, namely, not more than 10 days, and that birds are not allowed to exceed a period of 30 days upon the polished rice diet. The experiments also define the limits under which the test can be used with success.

PART II.

The response of the pigeon to a polished rice diet.

The quantitative behaviour of the rice-fed bird to test doses proves that each bird uses *per diem* a certain amount of this factor in the course of its metabolism. This amount appears to be a diminishing one, because animals which survive over the 30th day seem to require only small amounts of the active principle to keep them alive. At this stage the metabolism is shut down. Now the data in Part I show that there is a variation in the quantitative response amounting to some 40 % either way. This suggests that there is some difference in the amount of active principle required by different birds. If this be so, then it should be possible to show that different birds react to a polished rice diet differently. A bird which gives a longer number of days in the tests for the active principle should also take longer to develop symptoms upon the polished rice diet. This has been considered briefly in Part I, but no suggestions upon the matter can be entertained unless it prove to be a fact that birds react constantly to the polished rice diet, so far as the number

of days required to reach symptoms are concerned. Accordingly a search through the accumulated data of the last 3 years has been made (involving some 2000 cases). Records have been found of 63 cases in which birds have been used for tests 3 times or more, in 20 of these the birds having survived

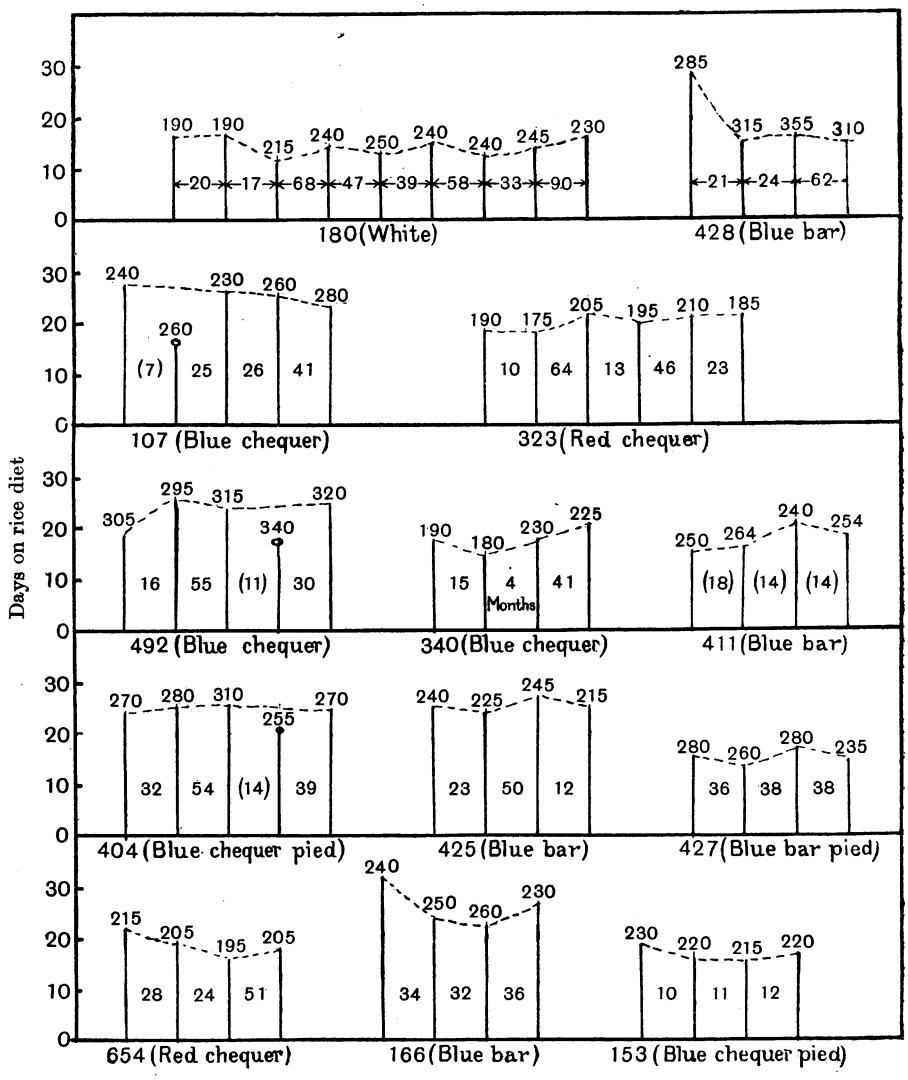

Fig. 1.

A. Vertical lines represent the length of time on rice before developing symptoms.
B. Numbers between the vertical lines represent the number of days interval on normal diet between any two experiments.
C. Numbers at the top of vertical lines show the weight of the bird upon the day upon which symptoms developed.

more than 3 test periods. The data were only collected as an incident in the testing work and in some cases cannot be cited owing to the absence of essential points. There are however enough complete records to deal with

the issue raised. Does an individual bird always develop symptoms on or about the same day after the commencement of rice feeding?

The chart (Fig. 1) gives the essential data for 13 cases in which birds have been used for experiment more than 3 times. The cases in which birds have survived only 3 treatments are given separately in Table VI. Upon the chart are recorded the colour of the birds, the number of days before appearance of symptoms (reckoned from the day upon which the bird was placed upon polished rice), the number of days interval of ordinary feeding between the experimental periods and the weight of the birds in each case at the onset of convulsions. The conclusions from Part I suggest that birds would react irregularly upon the first period of feeding, because most of the results here recorded were undertaken before recognition of the necessity for a preliminary period of standard feeding. Such an irregularity can be observed in the case of birds 428, 492, 425 and 153 and Table VI. Excluding this first period of feeding, the majority of birds show symptoms of head retraction on or about a day that is rather constant for the bird. For birds 323, 425, 428 and 153, the day for each bird may be regarded as a constant. The most interesting case is that of 180. This survived a period of experiment of more than 18 months, during which there are records of 9 sessions of rice feeding. During this time, the extremes of variation shown by the bird were from 11–17 days, being respectively 16, 17, 11, 14, 12, 15, 12, 14, 16 days. Of these probably 11 should be discarded, because the notes show that the bird was replaced upon the polished rice diet again after the short interval of 17 days upon a normal diet. Several cases of this error have been brought to light during an examination of the notes. In practically all of them the reaction time to the diet has been low and out of harmony with the remaining results. For instance, through some clerical error 107 was replaced upon polished rice after only 7 days of mixed feeding. It gives a response quite out of keeping with the other responses for the same bird. The same effect is seen in 404, 492 and 340. These figures have been excluded from the computation as the bird has evidently not had time to replenish its stores of vitamin. This constancy in the reaction time to a polished rice diet for a given bird was rather unexpected. The point receives strong support from the data upon the three-period cases in Table VI. Out of 41 cases found, 14 have had to be discarded owing to inadequacy of data. The remaining 27 cases appear in the table. It will be seen that 17/27 showed a difference between the second and third responses of not more than one day. Five of the remainder showed deviations of 2 or 3 days. Seeing that responses have varied over a period of 14 to 28 days, it is clear that these correspondences cannot be accidental. Further it is interesting that in the case of 600 and of 634, the high results given in the third figure are out of step with the first and second periods, and oddly enough both came from birds which had been put down upon the same day, April 14, 1927. This suggests that there may have been some error or otherwise in the feeding of this batch.

Table VI. *Three-period cases.*

No.	Colour	Average weight of last two weighings g.	Periods of feeding (days) First	Second	Third	Differences 2nd and 3rd	Day constant
108	Red chequer pied	245 ± 5	28	19	19	None	19·0
217	Blue chequer pied	215 ± 5	16	20	20	None	20·0
229	Blue chequer	282 ± 2	14	13	15	2 days	14·0 ± 1·0
286	Blue chequer	287 ± 2	14	24	27	3 days	25·5 ± 1·5
339	Blue chequer	225 ± 10	23	28	27	1 day	27·5 ± 0·5
344	Red chequer	340 ± 10	16	16	15	1 day	15·5 ± 0·5
372	Blue chequer	278 ± 18	30	23	24	1 day	23·5 ± 0·5
409	Blue bar	275 ± 20	17	24	24	None	24·0
457	Blue chequer	265 ± 15	16	19	19	None	19·0
493	Blue bar	222 ± 2	21	23	24	1 day	23·5 ± 0·5
505	Blue chequer	265 ± 5	29	21	21	None	21·0
522	Silver mealy	215 ± 5	25	18	19	1 day	18·5 ± 0·5
568	White	252 ± 2	22	17	16	1 day	16·5 ± 0·5
566	Blue bar pied	215 ± 20	19	16	12	4 days	14·0 ± 2·0
600	Red chequer	362 ± 12	14	14	21*	7 days	Nil
634	Grizzle	245 ± 15	15	14	18*	4 days	16·0 ± 2·0
651	Blue chequer pied	297 ± 2	18	19	19	None	19·0
763	Silver mealy	267 ± 2	24	19	26	7 days	Nil
684	Blue chequer pied	285	17	20	21	1 day	20·5 ± 0·5
709	Mealy	234 ± 6	29	19	22	3 days	20·5 ± 1·5
722	White	245 ± 10	23	15	14	1 day	14·5 ± 0·5
726	Blue	254 ± 2	18	19	23	4 days	21·0 ± 2·0
741	Mealy	300 ± 5	21	18	18	None	18·0
841	Red chequer	317 ± 12	22	24	21	3 days	22·5 ± 1·5
814	Dark blue chequer	224	24	23	26	3 days	24·5 ± 1·5
869	Blue chequer	250	24	18	18	None	18·0
473	White and blue	300 ± 10	26	17	18	1 day	17·5 ± 0·5

* Apr. 14.

The data give the clear-cut answer that the majority of birds react to the diet in a constant manner. Birds such as 166, Fig. 1, are an exception in not conforming to a general rule. This constancy of reaction to the diet is felt to be of fundamental importance. For an individual bird there tends to be a definite time for which that bird can subsist upon the diet and this time differs for different birds. Birds can be classified into "quick reacting" under 17 days, "average" 17–23 days and "slow" over 23 days. Table VII is an abstract of day constants grouped under the colours of the birds.

Table VII.

Colour	Number of days
Blue chequer	14, 25·5, 27·5, 23·5, 19, 21, 24·5
Blue chequer pied	20, 19, 20·5
Blue bar	24, 23·5
Blue bar pied	16
Red chequer (brown)	15·5, 14, 21
Silver mealy	18·5, 23
Grizzle	16
Mealy	20·5, 16·5
White	17·5, 16·5, 14·5

There seems here to be a tendency for the colours other than blue to react more quickly, though this is clearly not a general rule. The results for the blue chequer birds show that there is no reason for attributing slow and quick reaction to the colour. There is also clearly no correlation with the

weight[1]. The thesis of Abderhalden [1923, 2] and Grey [1927] that colours other than blue and black are not sufficiently resistant is not supported by this table. We have noted, however, that fancy birds such as the fantails make unsatisfactory cases.

We are now in a position to ask the further question, namely, whether it can be shown that those birds which react quickly to the polished rice diet give lower values for the test than others. It will be recalled that earlier in this communication it was stated that there was no correlation between the length of time of protection after cure and the day upon which the birds showed symptoms. Reliable evidence upon this point is somewhat scanty; the most reliable, which we have, would seem to be that included in Tables III and V. In Table III (B), the majority of the birds were reacting well to their "day constant." Yet the days of protection and cure do not correspond with the "day constants." This might be attributed to some toxic effect of the metals in the preparation, some birds being more susceptible to inhibitory influences than others. However, this objection is not present in the results of Table V which were obtained upon birds that had been previously standardised by the new technique. Here we have two cases both of which give an answer of 7 days to 0·2 cc. of the preparation, yet one of them has shown symptoms upon the 18th and the other upon the 28th day. Undoubtedly more evidence upon this point is needed, but what evidence exists is against the view that the variations in the test responses can be explained by variations in the "day constant." The puzzle raised by this finding will be discussed later. It may be concluded from this part that after a previous standardisation birds react in a constant manner to a polished rice diet. This constancy is not related to the colour or to the weight of the bird, nor does it explain the variations in the response of pigeons to test doses.

Summary of the variations which may affect the test.

The following is a summary of all the factors which have been found so far to affect the pigeon test, together with the means of avoiding them so far as they are known.

Gross errors. The dose may not be introduced completely, owing to constriction in the throat of the bird. The dose may be vomited by the bird. These errors can usually be excluded.

Errors of feeding. Birds should be standardised as regards diet before being placed upon polished rice for feeding. It would be doubtless better to have a standard variety of pigeon, but until such is available it is simpler to use birds at random from the open market.

Possible cures other than that due to the curative substance. The "heat" cure. This can be avoided by leaving birds in the warm for at least 2 hours before treatment.

[1] Unfortunately, data as to sex and age are not available. Pigeon 180 however suggests that age is not the determining factor.

The "glucose" cure. This can be avoided by giving a small dose of glucose in water, at the same time that the bird is being tested for "heat" cure. This also has the advantage that any cure producible by distilled water alone is also eliminated [Peters, 1924, p. 859].

Note. The "spontaneous" cure [Kon and Drummond, 1927] has been shown by Kon [1927] not to occur upon a polished rice diet.

It is believed that when these variations are eliminated the number of abnormal cases is reduced to a minimum and is not sufficient to disturb the tests. It is of course tacitly assumed here that the conditions previously laid down are observed, such as the administration of the test dose within 6–12 hours of the onset of symptoms, the elimination of any bird which appears to be too weak for the test, and of all birds which do not show the characteristic symptoms of head retraction within 30 days. A few birds show "forward" symptoms (emprosthotonus of McCarrison [1921]). These we have used occasionally when we have been pressed for test birds, but we have not been able to place much reliance upon the results of the tests. When the sources of error summarised above have been eliminated, we believe that the pigeon "curative" test is rapid, useful and accurate. In the Appendix is described in full the technique used in this Department.

Discussion.

It is hoped that sufficient evidence has been presented to make it clear that pigeons can be used for curative tests under stated conditions. The most interesting point that emerges for discussion is that birds have an individuality so far as their response to the vitamin-free polished rice diet is concerned. It has been shown that this is not a matter of colour or weight. The explanation must therefore be sought elsewhere. Not much information has been found in the literature upon this matter. With reference to fowls, Eijkman and van Hoogenhuize [1916] state that one of the factors which determines the length of the incubation period is the body weight at the beginning of the experiment. Vedder [1918], in confirmation of earlier work, concluded that the onset of polyneuritis in the fowl bore no relation to the amount of food eaten, but was a matter of the idiosyncrasy of the animal. The view that there is no relation between the time of onset and the amount of the food eaten is not generally accepted [see Braddon and Cooper, 1914; Funk, 1919; Plimmer, Rosedale, Raymond and Lowndes, 1927; Cowgill and Klotz, 1927]. It seems to be held rather widely that there is a relation between the metabolism and the requirements of the vitamin B complex. Using autolysed yeast as a source of vitamin, Collazo and Funk [1924] state that each pigeon requires a different amount of autolysed yeast to keep it in vitamin equilibrium. Their criterion for equilibrium is the maintenance of a constant weight. An examination of their table (p. 190), shows that the heavier birds ate more, and required only 80 % as much vitamin as the rather lighter birds. Summarising the above, it may be con-

cluded that previous observers have noted idiosyncrasy in the animals, but so far as can be ascertained no one has found that the majority of birds can be brought into a condition in which they react to the diet in a constant number of days. There seems to be no explanation at the moment of the idiosyncrasy. We are all aware of marked differences in man in appetite which are not obviously correlated with size or with the degree of adiposity. With regard to the "day constant" for a given bird, it is natural to assume that this represents a day upon which the vitamin store has become depleted in some sensitive part of the nervous system. If it were due to a daily diminution in the stores of vitamin, then a bird which is "quick reacting" should utilise vitamin faster than one which is "slow reacting." A curative dose should last the latter bird longer than the former one. This has been shown in Part II not to be the case, so far as the evidence is available. It is difficult to be quite certain that in these curative tests due allowance has been made for the possible effect of inhibitory factors. These might operate more powerfully in one bird than in another. But the data suggest that the day constant is not merely determined by the daily loss of the curative substance. Some other variable appears to be concerned. This may be the amount of food eaten by the bird, a matter which it should be possible to determine experimentally.

From the standpoint of the curative test, all that is necessary is to make certain that a bird under test is not liable to curative influences other than that of the test extract. It would be of assistance to have some coordinating theory to cover all the cases of cure, of whatever nature this may be. Attempts have been made, especially by Hess [1921] and Abderhalden [1923, 1], to explain the symptoms as due to some failure in the oxidations of the body. That this explanation cannot be applied to the tissues as a whole has been amply shown by the work of Roche [1925] and of Drummond and Marrian [1926]. The latter could find no difference between the tissues of the normal and avitaminosed pigeon as regards oxygen uptake or power of reducing methylene blue anaerobically. They also showed that the lowering of temperature in avitaminosis in the rat is connected with the lowering of body oxidations, which could be restored to normal by raising the temperature of the body as a whole. This proves that there is no general interference with oxidations. But the symptoms of opisthotonus are likely to be connected with a comparatively small part of the nervous system, changes in the oxidation of which may not show up experimentally when merged in the general metabolism. The most hopeful means of coordinating the data seems to lie in the view that the symptoms appear as the result of some local interference with the cycle of metabolic changes (probably of carbohydrate [Collazo, 1923]). Such interference may be produced by lack of a suitable catalyst. It may on the other hand be produced by failure of circulation, causing lack of substrate in the tissue or interference by accumulation of waste products. Histamine, heat, sugared water may all be means of restoring a failing circulation,

which has led to interference in function even in the presence of an adequate concentration of the active principle.

Appendix.

The technique.

The pigeons are standardised upon receipt from the dealer by placing for periods of 20 days to a month upon a mixed diet in an open-air cage of dimensions 6 ft. high × 6 ft. × 8 ft. The mixed diet has the following approximate composition, buckwheat 1 part, dari 1 part, split maize 1 part, split wheat 1 part and wheat 2 parts.

The pigeons are transferred in batches of 15 to cages of the dimensions 4 ft. 6 in. high × 2 ft. × 2 ft. These cages are fitted with wire bottoms and are cleaned thoroughly every 3 days to prevent the accumulation of excreta. The birds are then given a diet of polished rice (Patna)[1]. The polished rice is prepared by washing for 3 hours with running water, constantly stirring. It is drained, dried before the fire, and then autoclaved at 120° for 2 hours. The cages stand in the indoor animal house at a temperature of 10–15°. When the birds develop symptoms or appear to be about to do so, they are brought into the warm room, temperature about 21°. Here they are placed in smaller cages. Care is taken that these cages should not come into contact with birds which have not been upon a vitamin-free diet for some time. The birds are given a dose of about 50 mg. of purified glucose in 5 cc. of water and left in the heat for 2–3 hours before treatment in order to eliminate the various sources of extraordinary cure. The doses are introduced into the crop with a pipette fitted with a short length of bicycle-valve tubing.

At the conclusion of the experiment, the bird is given a dose of marmite, and placed in a special cage, where it is kept in the warm for 2 or 3 days until completely recovered. The bird is then restored to the open-air cage for a period of 20 days to a month before being subjected again to the rice diet.

Summary.

1. The evidence for the quantitative nature of the curative pigeon test has been examined. Reasons are given for the belief that the test can be used with confidence, and the conditions necessary for good results are enumerated.

2. Abnormal cases sometimes appear in which birds require much less than the average dose to cure and protect them from a further onset of symptoms. This is due in part to the influence of the previous period of feeding, and can be largely avoided by standardisation upon a laboratory diet before rice feeding. Such a period of feeding also improves the number of cases showing the characteristic symptoms before the 30th day.

[1] Birds are allowed to feed voluntarily throughout. Lumps of salt are also placed in the cages. Oxford tap water is given for drinking purposes.

3. Some birds have been cured by giving small doses of purified glucose. This "glucose" cure must be distinguished from the "heat" cure. As a routine each case may be given a dose of glucose at the time that it is placed in the hot room. The above procedures tend to eliminate the abnormal cases.

4. Variations of $\pm$ 40 % may be expected when pigeons are selected at random for the tests.

5. It is found that a majority of birds react constantly to the rice feeding, there being a constant day upon which the symptoms tend to appear, reckoned from the beginning of the rice feeding. This is termed the "day constant" for the bird, and may vary with different birds from 14 to 28 days. It is not correlated with the colour of the bird, or the weight at onset of convulsions. Birds can be classified into "quick" reacting and "slow" reacting.

6. Variations in the day constant do not explain the $\pm$ 40 % variations in the responses to test doses.

7. A summary of methods of feeding pigeons in this Department is appended.

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