CCVIII. MAINTENANCE NUTRITION IN THE ADULT PIGEON, AND ITS RELATION TO TORULIN (VITAMIN B_1). I.

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OUR interest in this subject arose from the desire to meet possible objections to our original contention that a curative and protective vitamin B_1 preparation would not give maintenance nutrition [Kinnersley and Peters, 1925]. We found it impossible to effect this completely without a more elaborate investigation (of which this paper forms part) into the factors needed to give maintenance nutrition in the normal pigeon. Accurate knowledge upon this is essential for the use of weight maintenance tests with pigeons in the assay of vitamin B_1 and also for a study of the symptoms of avitaminosis. As pigeons upon a polished rice diet are suffering, in addition to deficiencies of protein and salts, from a deficiency of more than one B factor, the pathology of avian polyneuritis cannot be properly understood until single instead of multiple deficiencies can be experimentally produced [Peters, 1929, 1930]. We need not stress the wider consideration that a full knowledge of the necessary factors, as they present themselves in various animals, seems to be fundamental to the study of human beri-beri. In this paper, B_1 and B_2 have their accepted meaning. The term B_3 is used for the pigeon factor [Williams and Waterman, 1928] and B_4 for the rat factor [Reader, 1929].

HISTORICAL.

A survey of the literature upon vitamin B shows much disagreement as to whether polished rice with addition of vitamin concentrates is sufficient to constitute a complete diet. Schaumann [1914] for instance said that this was not so. We find in the earlier work that there were attempts to compare the curative activity of vitamin B extracts with their powers of maintaining weight in the adult pigeon. An especially valuable study in this connection was made by Cooper [1913], who graded certain foodstuffs according to their power to prevent polyneuritis, and then found that this grading did not hold for their capacity to protect from loss of weight. Such evidence was very suggestive of two factors, but could not be taken to prove this, because it could be interpreted in other ways, as *e.g.* by the presence of inhibitory substances in some of the foods tested. Somewhat later Funk [1914] concluded that polished rice plus vitamin B_1 (a preparation of about 1 mg. per day activity) constituted a complete diet, though this conclusion does not stand out firmly, as he alludes in the same paper to another preparation which he obtained from yeast, which was curative but not effective in complete nutrition. Williams and Seidell [1916] found that the curative and weight-maintaining powers of vitamin B concentrates could be separated, but were inclined then to attribute this to the decomposition of the vitamin. Emmett and McKim [1917] showed clearly that supplements of vitamin adsorbed on fuller's earth and Lloyd's reagent would induce a rise in weight if given in sufficiently large doses, but that there was a quantitative difference between the amounts required to cure and to give full nutrition, when the original yeast extract was compared with the concentrate [see also Chick and Hume, 1917]. Kellaway [1921] found that polished rice with a daily addition of 1·0 g. of marmite led to an average daily gain of 1·7 g. over a period of 1 month, additions of 2·0 g. did not increase this gain, whereas 0·75 g. under the same conditions led to a gain of only 0·5 g. *per diem*. Plimmer and Rosedale [1923] found that 3·0 g. marmite to 90 g. rice + 15 g. fish meal gave maintenance of weight in some birds. Tsukiye [1922] using a vitamin concentrate from rice polishings found that it caused a gain in weight in hens feeding upon polished rice. Dissatisfaction with pigeon curative tests led to search for a new technique and the simple problem as to whether the vitamin was the only supplement needed to complete a polished rice diet was begged by the use of a weight maintenance test for vitamin B, which was introduced by Seidell [1917, 1922]. In this test, pigeons fed upon polished rice are given daily doses of vitamin B concentrate, and the amount of vitamin is gauged by use of the fact that weight is maintained practically constant if sufficient is present. This method was also adopted by Funk and Paton [1922] and by R. R. Williams, Eddy and their co-workers. Its simplicity as compared with the curative tests led to a rather wide adoption and to the belief that a substance could only be considered to be vitamin B if it were capable of preventing polyneuritis as well as inducing maintenance of weight upon a polished rice diet in pigeons. This conclusion was reached, it must be noted, in spite of the observation by Seidell [1921] that certain silver-baryta fractions were potent as curative but not as weight-maintaining substances. Abderhalden and Schaumann [1918], on the other hand, seem never to have accepted the view that the curative and weight maintenance factors were identical [see also Berg, 1923].

In 1925, after two of us had demonstrated to our satisfaction that the curative substances as such could be fractionated and concentrated to a high activity, we used up the remainder of the preparation then in our hands in an attempt to induce with it maintenance nutrition in pigeons and prophylaxis against polyneuritis. In this way it was found that two curative day doses of torulin (vitamin B_1) administered daily to pigeons upon a polished rice diet did not protect against loss of weight, though they protected against polyneuritis for over 30 days. Larger doses of the same preparation however after considerable loss of weight had occurred gave in one case a weight maintenance, but without storage of the vitamin for any dose other than the last dose administered. In so far as the vitamin concentrates of Seidell [1917, etc.], Funk, Harrow and Paton [1923], etc., maintained weight when given as supplements to polished rice, we drew the conclusion that more than one factor must be needed, and that vitamin B must be dual. Our experiments did not satisfy us completely. We had relied upon the statements of others that yeast extracts would support a maintenance nutrition. This might not have held under our conditions. Further there was always the question as to whether we had administered enough for maintenance. It was possible that more than twice the curative dose should be given each day to induce maintenance. As soon as larger amounts of the more active torulin preparations were available, we returned to the question. Jansen and Donath [1926] showed that their rice vitamin B would support a weight maintenance in pigeons only when supplemented with $2\frac{1}{2}$ % of meat powder daily and cod-liver oil; in this case they considered that the meat powder was supplying a deficiency of protein. The idea that the meat supplied some missing protein factor did not agree with previous work, because whatever was the supplement needed in the meat must have been present also in Seidell's vitamin B concentrates. Williams and Waterman [1928] described a factor (vitamin B_3) necessary for pigeons to gain maximum weight, as distinct from maintenance at some intermediate weight. This was not present in certain antineuritic yeast concentrates, though it was present in whole yeast and in whole wheat. These facts have been confirmed: marmite, a yeast preparation, containing vitamins B_1, B_2 and the Reader rat factor B_4 [Reader, 1929], will not induce more than a maintenance nutrition even when supplemented with caseinogen and salts; but rise to maximum weight takes place readily upon giving whole wheat [Peters, 1929]. In the Harben lectures [Peters, 1929], we stated that 3 day doses of a torulin preparation, given daily from the start of a feeding, did not induce maintenance of weight at any stage of nutrition. The fact that marmite gave maintenance and did not appear to contain the Williams and Waterman vitamin B_3, we explained by postulating the need of a supplement to vitamin B_1 for maintenance. But later Guha [1929] stated that adult pigeons upon vitamin B_1 preparations from wheat germ and brewer's yeast showed maintenance, or nearly so, with doses equivalent to 5–6 curative pigeon doses daily. Our proof therefore did not really cover this point, though the three doses given would have been for many pigeons equal to 5 (we chose the lowest figure given by our birds). This matter has now been settled. A preliminary account of our work has appeared [1930], and at the same time there came into our hands through the courtesy of Prof. Eddy the papers by Eddy, Gurin and Keresztesy [1930], and by Williams, Waterman and Gurin [1930]. In the latter paper the authors show that the Jansen and Donath vitamin is protective to pigeons against polyneuritis, but does not give maintenance nutrition. They have therefore reached by a different route essentially the same conclusions as ourselves, though the fact that their preparations are not standardised in the same way makes it difficult to decide whether the point raised by Guha has been covered in their work.

In order to prove that vitamin B_1 does not alone give maintenance in pigeons feeding upon polished rice, it must be shown that an amount of vitamin B_1 adequate for maintenance in an impure yeast extract does not suffice when a purer vitamin is given instead. So as to make certain that variations in birds are not influencing results, the purer vitamin B_1 must be given in excess of the largest amount which normal birds require. In our case all the extracts have been standardised by the same method, and we have used as a basis a standard preparation of crude marmite. Our experiments have been planned as simply as possible in order to narrow the issues. Polished rice has been supplemented with the crude yeast extract: we have then tried to imitate the effect of this crude yeast extract by various torulin preparations. We shall first describe what we mean by maintenance nutrition, and then show in confirmation of Kellaway [1921] that a supplement of approximately 1·0 g. of marmite will produce maintenance for quite long periods. There will then follow the evidence that large amounts of yeast vitamin B_1 do not imitate marmite as supplement; this is a rigid proof of our former point, and shows that vitamin B concentrates which induce weight maintenance (such as that of Seidell) must be composite. The next question arising is how much vitamin B_1 is wanted to give weight maintenance. This involves a knowledge of the other factors supplementing B_1, and has not yet been settled.

Experimental.

Over 100 pigeons have been used during the course of this research. We have gained much from a continuous study of 15 pigeons over a period of 20 months. The birds have been kept upon screened bottom cages in groups in a room of temperature varying between 10 and 18°. We have run control experiments, but have not been able to find that variation in temperature affected the results. Pigeons have been fed upon polished rice and tap water with doses every third day of cod-liver oil. The polished rice has been treated by our usual methods. Birds have been weighed at 9.30 a.m. and doses of curative extract given into the crop at 4 p.m. Where more than one extract had to be dosed to the same bird, the extracts were given separately. In all cases the birds have been allowed to feed naturally, and no attempt has been made to check the amounts of food eaten. In spite of the rather sweeping remarks of Mitchell [1929] upon this point the amounts of food do not concern our facts as to weight and its maintenance.

Standardisation of the curative extracts.

Since the proof below depends ultimately upon the methods, these require discussion. That of Kinnersley, Peters and Reader [1928] has been used throughout, and the average figures for the preparations are given below. We have given the outside figures of the deviation from the mean in the case of the marmite and of the X preparation, so that we can exclude the possibility that the figures upon which reliance is placed in this paper are vitiated by the selection of birds which lay upon the outside limit of our averages.

Marmite. This has been used as a standard in the form of the crude extract supplied by the company. The curative figures for 1·0 g. marmite were in days, 4, 3, 4, 4, 4, 6, 3, 7, 4, 6, 3, 4, Av. 4·3. The significant figures are the average 4·3, and the maximum of 7. This it will be seen is quite exceptional.

Torulin. It has been our custom [Kinnersley and Peters, 1928] to make two extractions of the charcoal, one by aqueous HCl (X preparation) and the other by alcoholic HCl (Y preparation). The X preparation is purer, and as we shall show has different properties.

X preparation. Out of a series of birds this reacted very constantly at 4·0 days per 0·1 cc. One bird gave 3 days.

Y preparation. The figures here given for the average of 3·0 were the average of 37 tests. It has been our experience that the tests with this preparation are not so consistent as those with others. We do not profess to understand this at present, but merely record it as our impression that the deviation from the mean curative dose is greater with some preparations than with others.

Methods of preparing the concentrates used in these experiments.

X preparations. $N/10$ HCl extract of the charcoal. This material was obtained from 112 lbs. of baker's yeast, by the method of Kinnersley and Peters [1927, p. 785]. After boiling off H_2S and reducing to a volume of 40 cc., the solution was fractionated with alcohol by the method of the same authors [1928, p. 426], the fractionation being carried to the absolute alcohol stage, with a change of reaction to p_H 3·5. After removing the alcohol, the residue of approx. 4·0 g. was dissolved in 150 cc. water, and sufficient HCl added to make acid to Congo red. It was then treated with 0·5 g. norite charcoal. The charcoal was separated and extracted with $N/10$ HCl to remove any adherent vitamin. To the combined filtrates, 115 cc. of phosphotungstic acid (10 % in 10 % H_2SO_4) were added, this amount being short of that required to produce maximum precipitation. The phosphotungstate was decomposed by grinding with solid baryta, excess Ba removed from the filtrate with sulphuric acid, and the reaction brought to Congo red acidity with HCl; NaOH was then added to p_H 3·5–4·0, and the solution concentrated first on the water-bath and then *in vacuo* at a low temperature. It was then fractionated until soluble in 99 % alcohol, giving torulin, which remained stable in alcoholic solution for over 2 years, of an activity rather greater than 0·1 mg. per day.

Y preparations, prepared from the 50 % acid alcohol extract of the charcoal. Two of these were used, A and B. (A) was made from baker's yeast by our usual methods; (B) was a mixture of a preparation from baker's yeast, and of one from a British Drug Houses concentrate. They were both fractionated by a new phosphotungstic method, shortly to be described in detail. In view of the lengthy description which would be entailed if all the experimental details were given in the latter case, we propose to make only the essential points clear[1].

(A) Preparation from 56 lbs. of baker's yeast. The details described by Kinnersley and Peters [1927, pp. 785, 786] were adhered to with the following exceptions. The yeast was extracted upon arrival at the laboratory. At the baryta stage, a small volume of hot baryta solution was used to precipitate the gum, instead of the large volume of cold solution. We have used this modification of the original process now for some time, as we have found that it greatly diminishes the volume at the final stage, and therefore facilitates the manipulations.

After extracting the charcoal with $N/10$ HCl, the 50 % acid alcohol extract was made as usual. The acid alcohol extract of 3 litres was concentrated to 200 cc. on the water-bath after addition of NaOH to make purple to Congo red. Water was then added to 400 cc., the solution brought to p_H 3·5 and H_2S passed for 3 hours. The sulphide was suspended in acid water and H_2S again passed. The p_H of the combined filtrates was brought to 2·5 approximately, and the H_2S removed by boiling. To the volume of 460 cc., 70 cc. absolute alcohol were added and HCl to p_H 2·0 (turn of thymol blue). The extract was kept in cold store. Activity 3·1 mg., 6300 doses.

(B) This was a mixture of a preparation made from (1) 51 lbs. of baker's yeast and (2) B.D.H. concentrate. (1) was carried out to the charcoal stage by the usual methods with the exception that the yeast was allowed to stand for 12 hours in 16 litres of water before extraction. After adsorption upon the charcoal, the stage of $N/10$ HCl extraction was omitted. A 50 % alcohol acid extract was made, concentrated, and then fractionated twice by our newer phosphotungstic methods, and finally with alcohol. Activity 0·16 mg., 2900 doses. (2) Concentrate purchased from British Drug Houses. This concentrate is said to be from distiller's yeast. As supplied it is stated that 1·0 g. equals some 20 g. of the original yeast. As we have not yet given the details of working up this material, as far as the charcoal stage, we shall give these in greater detail. The main advantages are that work can be carried out with smaller volumes than when the initial step is the yeast itself, and the laborious stage of extraction is avoided. Against this has to be set the disadvantage that the commercial preparations are apt to be variable in activity.

5 lbs. of the concentrate (equal to 90 lbs. of the original yeast) were diluted to 24 litres. 2800 cc. lead acetate (25 % neutral) were added, and the precipitate was removed by filtration. The filtrate was then treated with sufficient hot baryta to precipitate the gum (this step being the same as in the case of the ordinary preparations), and the excess barium removed with sulphuric acid. The solution was brought to p_H 3·0 and 1100 cc. 10 % mercuric sulphate

[1] Should the experimental details be needed, we shall be happy to supply them.

solution (Hopkins) stirred into it, the large precipitate being removed by filtration. The filtrate was brought to p_H 7·0 and treated, in all, with 900 g. of norite charcoal. Two extractions of the charcoal were made first with $N/10$ HCl and then with acid alcohol. In the particular case under consideration the $N/10$ HCl stage was omitted, the charcoal being extracted five times with 50 % acid alcohol, about 6000 cc. being used. The extract was concentrated to 1060 cc. This was treated with 5·0 g. of charcoal, and the filtrate extracted three times with ether. The ether was removed upon the water-bath, volume 400–500 cc. At this stage it was fractionated twice with phosphotungstic acid by the new methods. When combined the above fractions gave 6000 day doses of torulin (approx. 0·2 mg./day).

Maintenance nutrition.

Several nutritional conditions can be recognised in pigeons. There is a weight minimum which is about 60 % of the weight maximum. There is also a rising, falling and maintenance nutrition, the terms being self-explanatory. Weight maintenance may be defined for our purposes as a condition in which the weight remains constant over a period of 30 or more days, with a continuous daily rise or fall of not more than 1·0 g. Work upon this problem has convinced us that it is necessary to distinguish these conditions as in some sense separate nutritional states. For instance, it requires less of a yeast concentrate to keep a bird at a weight near the weight minimum than at other weights. There is another possible complication, which we wish to exclude from discussion in this paper. It has been our experience in dealing with pigeons for curative tests that some difference in the bird appears after feeding upon polished rice for more than 30–35 days. Others seem to have felt this too, because they have also chosen birds for test within this approximate period. In order to narrow the issue here, we propose to limit our attention to pigeons which have been nourished upon the following diets within 35 days of the termination of the experiment, namely whole wheat, mixed grain, or polished rice supplemented with marmite.

In our experience the weight maintenance, which is so characteristic a feature of the work of Seidell and others, can be produced at most stages of nutrition between maximum and minimum in pigeons feeding naturally upon polished rice by adding to the diet doses of marmite given by tube into the crop. This is a remarkable happening from a physiological standpoint. Fig. 1 is typical of such a maintenance at intermediate nutrition. It shows the preliminary fall upon polished rice, the maintenance with an addition of 2·0 g. of marmite autoclaved at 115° for 1 hour (A), and the fall when the temperature of autoclaving was raised to 120° (B). In confirmation of Kellaway [1921], 1·0 g. of fresh marmite daily is sufficient in most pigeons to give maintenance. Kellaway found a slight rise, but this may well have been due to differences in the marmite preparation. Fig. 2 shows the effect of increasing the amount of marmite from 0·75 g. *per diem* up to 1·0 g., 2·0 g. and finally

4·0 g. A marked rise in weight takes place which tends to become stationary, and is never the equivalent of that obtained with whole wheat; the weight stops short much below the possible maximum. It is probable (see Part II) that these rises in weight are induced by traces of vitamin B_3 still present in the marmite.

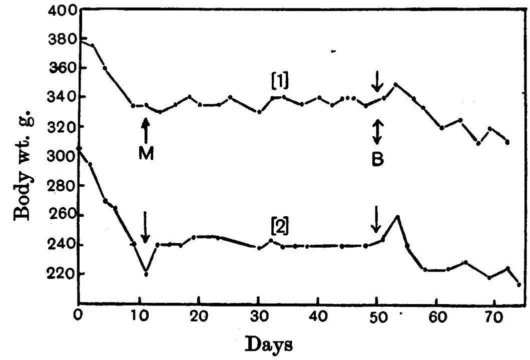

Fig. 1. Maintenance on marmite. Doses of marmite, autoclaved at 115° given at *M*. At *B*, diet changed to salted rice and marmite autoclaved at 120°.

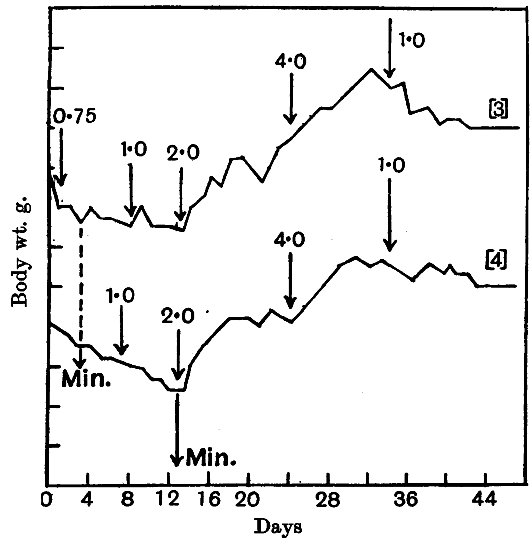

Fig. 2. Large doses of marmite. Ordinates. Divisions 20 g. Weight on 20th day, bird 3, 420 g.; bird 4, 385 g. Figures at arrows refer to daily doses of marmite in g. Minimum possible weight for bird 3 marked by dotted arrow.

Accepting 1·0 g. of our marmite preparation as the maintenance dose at an intermediate weight, we have found that such an amount contains 4·3 curative pigeon day doses. If vitamin B_1 is the only effective supplement in the marmite, then this amount of our concentrates standardised under the same conditions should imitate the dose of marmite. On the contrary, reference to Fig. 3 shows that 8–12 day doses of some of our concentrates (X) are not enough to give maintenance[1]. They are not even enough if we assume that in the marmite experiments we had selected the most sensitive birds, and in the others the least sensitive. If we had done this the figures would be 7 doses for marmite and 9 doses for the X preparation. In bird 6, transference to 1·0 g. marmite on 38th day readily gave maintenance. It is therefore proved that vitamin B_1 alone is not adequate for weight maintenance, and thus our former point is firmly established. In this matter, we find ourselves to be in complete agreement with the latest work of Williams, Waterman and Gurin [1930], who have used the Jansen and Donath vitamin from rice together with a protective test.

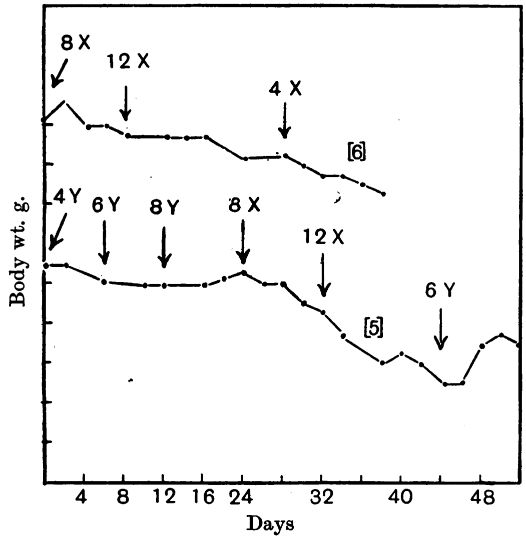

Fig. 3. Doses of vitamin B_1. Ordinates. Divisions 20 g. Weight on 24th day, bird 5, 375 g.; bird 6, 375 g. Numbers upon charts represent the number of vitamin B_1 doses of X or Y preparations.

How then are we to explain the results of Seidell [1926] and of Guha [1929]? We find that maintenance is produced by giving 6–8 doses of our 50 % alcoholic extracts of the charcoal (Y concentrates) (Fig. 3). Bird 5 illustrates this. These imitate the effect of the marmite. As little as 1·2 mg. of our purified Y preparation of vitamin B (YB) was sufficient to give weight maintenance. With this preparation there was a tendency for the weight to drop in some birds towards the 30th day of feeding. Since this "YB" was effective in such small amounts, and had been purified by phosphotungstic acid in addition to the charcoal adsorption, it is evident that the maintenance

[1] Some X preparations are not so free from the other factor. Out of all the birds and the various X preparations tested, we have never found a case in which 4–6 day doses were sufficient for maintenance. With 10–12 doses in the case of one bird upon one of the X preparations, the drop was not larger than 1·0 g. *per diem.* Even this was in contrast to similar doses of a Y preparation, which gave no loss or a slight increase in weight.

effect produced by marmite (in contradistinction to the fall with vitamin B_1 alone) is not due to the presence in the marmite of necessary protein or salt factors in the ordinary sense. The Y concentrate therefore contains some factor (or factors) in addition to vitamin B_1 needed by the pigeon for maintenance. It follows from Seidell's experiments and from our own that this factor tends to accompany vitamin B_1 in the processes of purification. Jansen and Donath [1926] found that their rice vitamin did not give weight maintenance in pigeons unless it was supplemented with an addition, which could be supplied in their case by meat powder thoroughly extracted with boiling water. They considered that this was a supply of protein. Our experiments with the Y preparation show that the factor which they were supplying was not protein in the ordinary sense, but one which can also be concentrated from an aqueous extract of baker's yeast. It is clear that we cannot settle how much vitamin B_1 a pigeon needs for maintenance until we can supply all other B factors in excess, and hence until any supplementary factors can be isolated. In the Harben lectures, we suggested that the factor needed as supplement might prove to be identical with vitamin B_4 which is known to be present in the Y type preparations[1]. Subsequent work which is being prepared for publication suggests that the supplement involved differs from previously characterised factors of the vitamin B complex.

Discussion.

This paper settles part of our original problem. Vitamin B_1 (from yeast) will not give maintenance nutrition in pigeons unless it is supplemented with some undefined factor. It is obvious, as pointed out by Williams, Waterman and Gurin [1930], that work upon vitamin B_1 in which maintenance tests upon pigeons have been used must be substantially revised. The contention [Kinnersley, Peters and Reader, 1928] that experiments such as those of Plimmer *et al.* [1927] must be interpreted as experiments upon the vitamin B complex is certainly justified. Until some chemical test is available, we are inclined to think that curative tests upon the pigeon or the rat [Kinnersley, Peters, and Reader, 1930; Smith, 1930] are our only reliable means of standardising foodstuffs for vitamin B_1 in spite of the rather large variations shown by such tests. The protective test is complicated by two considerations.

(*a*) It implies a knowledge of the time of onset of symptoms in the absence of vitamin B_1. In our experience this is a variable quantity in different pigeons. It might be possible to make use of our finding that individual pigeons show a surprising uniformity in the time at which symptoms appear. So far as we know, this has not yet been done.

(*b*) The second consideration is rather more serious. We have pointed out elsewhere (Harben lectures) that after the 30th day of rice feeding, pigeons appear to need more vitamin B_1, because 3–4 day doses of torulin given daily

[1] Reader (personal communication).

were not always sufficient to protect against symptoms. In their recent assay of the Jansen and Donath rice vitamin, Williams, Waterman and Gurin [1930] obtained a figure of 0·04 mg., a higher figure than that of the original authors' tests of 0·01 mg. *per diem*. We think that this is possibly due to the fact that they made preventive tests at the weight minimum, upon pigeons in the period after the 30th day of rice feeding.

We have refrained from discussing the most interesting feature of experiments of this type, the fact that these minute additions to the diet can enable the animals to balance intake and output so that weight is maintained for long periods within a few grams, at a point intermediate between the maximum and minimum weight of which the animals' economy is capable. A physiological adjustment is surely here indicated as precise as those which Haldane and others have shown to regulate the breathing.

SUMMARY.

1. In adult pigeons feeding naturally upon whole wheat or polished rice, with or without supplements of marmite or torulin concentrates, there can be distinguished a maximum, minimum or intermediate weight, and a rising, falling or maintenance nutrition.

2. A daily dose of marmite of 1·0 g. is sufficient to convert a falling nutrition upon polished rice into maintenance at almost any point between the maximum and minimum weight. This dose of marmite contains 4·3 day doses of vitamin B_1.

3. Large amounts of vitamin B_1 (up to 12 doses) given as the $N/10$ HCl extracts of the charcoal do not imitate the effect of the marmite. This establishes our former contention that purified torulin (vitamin B_1) does not give maintenance.

4. Maintenance nutrition is however given by 6–8 doses of our 50 % alcoholic extracts of the charcoal. These therefore contain some other factor necessary for avian nutrition.

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