

CXXXIII. MAINTENANCE NUTRITION IN THE PIGEON AND ITS RELATION TO HEART BLOCK.

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(Received May 5th, 1934.)

In a previous paper [Carter, 1930] it was shown that pigeon heart block which develops on a diet of polished rice could be cured if the birds received daily rations of whole wheat constituting not less than about 60 % of the total food intake. Vitamins B₁ and B₂ and the fat-soluble group of vitamins were found to be ineffective in restoring normal heart function when administered as supplements to the diet of polished rice. Heart block in the pigeon is therefore a condition of dietary origin. The question remains whether it is due to the deficiency of a specific factor as is the case in polyneuritis, or whether it arises from a deficiency of one or more of those factors which contribute to maintenance nutrition at maximum weight.

In some experiments to be described it will be shown that pigeons previously depleted on a diet of polished rice can, in a majority of cases, be restored to maximum weight by appropriate supplements to this diet without inducing any recovery of normal heart function. Substitution of an extract of hydrolysed wheat germ for the supplements results in a complete recovery from heart block without further significant change in weight. It will be further shown that birds previously brought to maximum weight on whole wheat can be maintained at this level on a diet which fails to protect them from the onset of heart block. The fact, previously recorded, that birds depleted on polished rice, when fed on whole wheat generally recover normal heart rhythm at a time when restoration of body weight is far from complete, has been confirmed. Finally the protein intake of birds on a polished rice diet has been examined, and its significance in relation to the problem of pigeon nutrition is considered.

Method.

Birds under test were kept singly in small cages in order to obtain measurements of the food intake. Observations on body weight were made at the same time each morning in order, as far as possible, to avoid fluctuations due to variations in the amount of food present in the crop. Heart rate and rhythm were noted at frequent intervals.

The source of vitamin B₁ employed was a yeast concentrate prepared according to the method of Kinnersley and Peters [1927] by the extraction of activated norite charcoal with 50 % acid alcohol¹. In all cases cod-liver oil has been given at regular intervals. Caseinogen (Glaxo, "Vitamin B-free") was

¹ This partially purified concentrate may contain, in addition to vitamin B₁, another factor, vitamin B₅, which has been suggested as being necessary for the pigeon [Carter *et al.*, 1930].

extracted continuously in a Soxhlet apparatus with 90 % alcohol for 48 hours and dried. It has been assumed in this paper that caseinogen treated in this manner provides a source of protein free from possible contaminating substances which might influence the nutrition of the pigeon. Before administration by pipette portions of 2 g. were weighed out, suspended in 20 ml. Ringer solution and brought to p_{H} 7.0 by addition of caustic soda. Hydrolysed wheat germ was prepared by heating for 3 hours with *N* hydrochloric acid and filtering hot on a Büchner funnel. This method, which differs from that employed by O'Brien [1934], was used daily to prepare the extract in order to avoid the possibility of its deterioration on standing in air.

EXPERIMENTAL.

The earlier experience of the effect of marmite, which in daily doses of 1.5 g. administered over periods of 30 days and upwards showed curative activity in regard to heart block in a certain proportion of cases, prompted a hope that the factor concerned might be fractionated from yeast. The negative results which have invariably been obtained with vitamin B₁ concentrate suggested that preparations from the precipitate which appears on treatment of an aqueous yeast extract with basic lead acetate might be effective. The results have unfortunately been variable and do not at present justify detailed consideration.

The lead precipitate, after treatment with sulphuric acid followed by hydrogen sulphide to remove all traces of lead, when administered to pigeons fed on polished rice is entirely without influence either in preventing decline in weight or in improving the heart condition. In an experiment in which this extract was supplemented by daily doses of an antineuritic concentrate together with 2 g. caseinogen 2 birds showed restoration of normal heart function within 20 days, while in another bird definite improvement though incomplete cure was noted. In all three cases there was gradual recovery over a period of 50 days to maximum weight. Repetition of this experiment with similar treatment resulted in complete cure of heart block in one bird, partial recovery in a second, and no improvement in a third. Restoration to weight maximum was observed in two of these birds while in the third the rise of weight, though substantial, failed to reach that obtained on whole wheat.

These preliminary experiments made it important to ascertain whether the induction and cure of heart block could be dissociated from factors associated with maintenance or restoration of normal body weight.

Effect of yeast concentrate and caseinogen on pigeon nutrition.

A group of 8 birds was placed on a diet of polished rice for periods varying from 12 to 24 days. During this time the usual fall of weight and onset of heart block occurred. In one case acute polyneuritis developed before treatment was commenced. There is at present insufficient evidence to indicate whether variation in the duration of the depletion period has any influence on the subsequent response. A control group of 3 birds was given daily doses of a vitamin B₁ concentrate in amounts equivalent to 12 units daily.

Table I and Fig. 1 show that the progressive decline in weight is arrested and that there is some rise which is less marked than that observed with birds receiving in addition a daily supplement of 2 g. caseinogen. This confirms previous experiments in which maximum weight has not been fully restored by supplements of yeast concentrate or marmite to the basal diet of polished rice.

Table I. *Effect of polished rice and 12 units vitamin B₁.*

| Bird | Maximum weight g. | Weight after depletion on polished rice | Duration of depletion days | Final weight | Duration of curative period days |
|------|-------------------|---|----------------------------|--------------|----------------------------------|
| 880 | 396 | 287 | 17 | 338 | 28 |
| 110 | 400 | 250 | 24 | 260 | 28 |
| 111 | 360 | 218 | 24 | 255 | 28 |

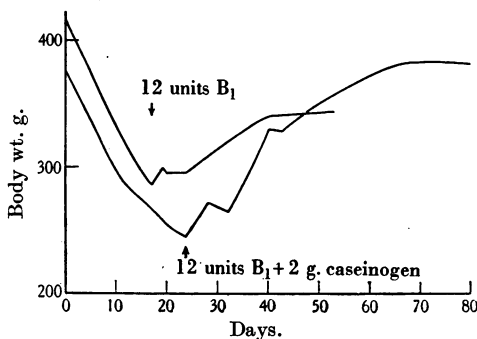


Fig. 1.

Table II records similar data for those birds on polished rice receiving daily 12 units of vitamin B₁ and 2 g. caseinogen.

Table II. *Effect of polished rice supplemented by 12 units vitamin B₁ and 2 g. caseinogen.*

| Bird | Maximum weight g. | Weight after depletion on polished rice | Duration of depletion days | Final weight | Duration of curative period days |
|------|-------------------|---|----------------------------|--------------|----------------------------------|
| 401 | 324 | 268 | 12 | 325 | 59 |
| 357 | 376 | 247 | 24 | 380 | 53 |
| 722 | 468 | 286 | 24 | 398 | 57 |
| 622 | 392 | 268 | 15 | 376 | 54 |
| 485 | 390 | 285 | 15 | 380 | 54 |

It will be seen that of these birds only one, No. 722, failed to reach maximum weight in the course of the experiment. In this respect their behaviour is in agreement with that of the birds in the earlier experiments.

In another experiment 2 birds, previously brought to maximum weight on mixed corn, were placed on polished rice supplemented by 2 g. caseinogen and 12 units of vitamin B₁ daily. After an initial fall of weight of 28 and 16 g. respectively during the first 4 days there was almost no change throughout the experimental period which lasted for 37 days (Table III).

Table III.

| | Day | Bird 622 | Bird 485 |
|--|-----|----------|----------|
| Initial maximum weight | 0 | 418 | 434 |
| Weight after commencement of experimental diet | 4 | 402 | 406 |
| | 10 | 405 | 411 |
| | 20 | 399 | 404 |
| | 30 | 390 | 396 |
| | 37 | 398 | 401 |

The rather unexpected result of these experiments raises a number of points of interest. In the first place the rise of weight to maximum is notably slow in comparison with the rise seen on whole wheat. Of the birds referred to in Table II full weight was not attained in two cases until the 40th day while in another it was delayed until the 59th day. This is about four times as slow as in the case of whole wheat. The fact that the weight rise is so gradual under these conditions raises the possibility that experiments of short duration may lead to misleading conclusions.

While the attainment of maximum weight upon a given diet may, subject to the possibility of storage of essential factors during the initial depletion period, be justifiably held to indicate that such a diet is "adequate" for full nutrition it does not necessarily follow that it contains a satisfactory balance of essential dietary constituents such as is present in whole wheat. In two respects a diet of polished rice may be ill-balanced. In 4 birds it was found that daily administration of 6 units of vitamin B₁ was inadequate with caseinogen, whereas an increase of dosage to 12 units sufficed to promote slow continued rise of weight. This large daily requirement of vitamin B₁ may be more apparent than real since the concentrate in which it is administered is also the source of vitamin B₅, another essential dietary constituent for maintenance in the pigeon, and it is possible that the latter may here be a limiting factor. On the other hand, as suggested by Williams and Eddy [1931], there may be a real difference in the requirement of vitamin B₁ for cure of polyneuritis and for maintenance of full nutrition. Morris [1933] claims that, with a basal diet of autoclaved wheat, the dose of a vitamin B₁ concentrate necessary to effect complete weight recovery after depletion exceeds the dose required for cure of polyneuritis by an amount which varies from six to sixteen times with different types of yeast concentrate. On the other hand where the basal diet is one of polished rice full recovery does not occur even if as much as 30 curative doses of vitamin B₁ are administered daily [Carter *et al.*, 1930]. The difference in the nature of the basal diet is important. The factor which appears to limit weight recovery on polished rice supplemented by a yeast concentrate is a relative deficiency of protein. Moreover a difference in the type of protein available on diets of wheat on the one hand and polished rice supplemented by caseinogen

Table IV. *Average daily intake.*

| | Bird 880 | | | Bird 110 | | | Bird 111 | | |
|--|--------------|------------|--------------|--------------|------------|--------------|--------------|------------|--------------|
| | Total calcs. | Protein g. | Days on test | Total calcs. | Protein g. | Days on test | Total calcs. | Protein g. | Days on test |
| Wheat | 79.23 | 2.50 | 9 | 72.34 | 2.29 | 11 | 80.95 | 2.56 | 5 |
| Rice with 5 units' vitamin B ₁ | 71.42 | 1.18 | 5 | — | — | — | — | — | — |
| Rice with 10 units vitamin B ₁ | 83.20 | 1.37 | 10 | 60.70 | 1.00 | 10 | 59.27 | 0.98 | 10 |
| Rice with 12.5 units vitamin B ₁ | 85.34 | 1.41 | 11 | 55.34 | 0.92 | 9 | 71.42 | 1.18 | 10 |
| | | | | Bird 622 | | | Bird 401 | | |
| | | | | Total calcs. | Protein g. | Days on test | Total calcs. | Protein g. | Days on test |
| Wheat | | | | 89.57 | 2.83 | 6 | 82.7 | 2.61 | 6 |
| Rice with 2 g. caseinogen, and 12 units vitamin B ₁ | | | | 85.0 | 3.15 | 52 | 87.8 | 3.18 | 13 |

on the other may perhaps contribute to the difference in the time required for weight recovery in the two cases. Table IV shows the calory and protein intake for birds on diets of polished rice supplemented by a yeast concentrate, with and without addition of caseinogen.

Birds 110 and 111 show some diminution in their daily calory intake as compared with that on whole wheat, whereas in the case of bird 880 this appears to be satisfactory. Sugiura and Benedict [1923] found, in experiments in which pigeons were forcibly fed on a diet containing 22 % caseinogen, that a daily intake of about 70 calories sufficed for normal growth and maintenance at full weight.

There is however in all birds on a rice diet a substantial diminution of the daily protein intake. On the other hand birds 622 and 401, which rose to their maximum initial weight on a rice diet to which were added 2 g. caseinogen in addition to the yeast concentrate, show daily calory and protein intakes which compare favourably with the intakes on whole wheat.

The biological value of the protein of polished rice has been regarded by Osborne *et al.* [1915] as high, but on the other hand its protein content is only about 50 % of that of whole wheat. There is at present no information as to the minimum protein requirement for the pigeon at maximum weight and the figures for protein intake on a polished rice diet do not therefore prove that this intake is inadequate. However, the fact that an additional protein supplement in the form of caseinogen to the basal diet enables the bird to regain full weight suggests that insufficient polished rice is consumed to meet its protein requirement. In this connection the observations of Aykroyd [1930], on the significance of partial protein deficiency in human beri-beri, may be mentioned.

It is desirable at this stage to define the sense in which the term "maximum weight" has been used in the preceding experiments. It is not here intended to imply that maximum weight represents some fixed and constant level of nutrition for any particular bird, since the body weight at any given moment is determined not only by the nature of the diet which it is receiving but also by its past dietary history and doubtless by other factors. The birds, before experiment, are placed on mixed corn for some weeks, and during this time come into equilibrium at a weight which has been here termed "maximum." If, after a period on an experimental diet having polished rice as its basis, the birds are returned to whole wheat the new weight which is established is not necessarily identical with the former maximum. In some cases, examples of which will be seen in experiments with wheat germ, the new weight level somewhat exceeds the initial "maximum." On the other hand a number of cases have been met with during the course of work in this laboratory in which whole wheat or corn fails to restore the full original weight level. It is not the purpose of this paper to discuss the factors underlying these variations in maximum weight. The facts must be borne in mind in assessing the significance of the experiments with caseinogen which have been described.

Effect of vitamin B₁ and caseinogen on pigeon heart block.

In the experiments described in the preceding section repeated observations were made on the condition of the heart. At the end of the initial depletion period on polished rice irregularity of rhythm and bradycardia, which in some cases was very marked, were observed. For example bird 722, after a depletion period of 24 days, showed a fall of heart rate from 200 per minute to 66 per minute.

It has been frequently observed that some rise of rate is associated with rise of body weight though the persistence of irregularity of rhythm shows

that the underlying disorder of the vagus centres remains. In all the birds whose weights are recorded in Table II heart block persisted through the entire period of the experiment in spite of a weight rise to maximum in four of them. Table V shows records of the heart in two typical birds of this group.

Table V.

| Days on experimental diet | Heart rate and rhythm | |
|---------------------------------|-----------------------|-------------------|
| | Bird 722 | Bird 357 |
| 0 | 66 Regular | 112 Dropped beats |
| 4 | 144 Dropped beats | 144 " |
| 9 | 132 " | 164 " |
| 18 | 144 " | 148 " |
| 26 | 128 " | 164 " |
| 38 | 148 " | 156 " |
| 54 | 128 " | 160 " |

In the experiment in which birds, having attained maximum weight on a diet of mixed corn, were maintained at this weight on a diet of polished rice supplemented by caseinogen and vitamin B₁ concentrate, heart block developed at the 8th and 15th days respectively and persisted through the experiment lasting 37 days.

These experiments show that normal nutrition as judged by weight tests can be established in conjunction with a dietary deficiency which is revealed by the presence of cardiac arrhythmia. Experiments now to be described show that this deficiency can be supplied by a constituent of wheat germ.

Effect of wheat germ on pigeon heart block.

Table VI shows the effect of administration of wheat germ hydrolysed for 3 hours in association with 12 units of vitamin B₁ concentrate.

Table VI.

| Bird | Before administration of wheat germ | | Weight g. | After administration of wheat germ (6 g.) | | Duration of treatment days | Initial maximum weight g. |
|------|--|---------------|--------------|--|---------------|-------------------------------------|------------------------------------|
| | Heart rate | | | Heart rate | | | |
| 622 | 140 | Dropped beats | 376 | 152 | Regular | 23 | 392 |
| 485 | 100 | " | 380 | 176 | " | 16 | 387 |
| 722 | 160 | " | 398 | 174 | " | 13 | 468 |
| 357 | 160 | " | 380 | 192 | " | 14 | 376 |
| | | | | Wheat germ 4 g. | | | |
| 940 | 124 | " | 253 | 160 | Dropped beats | 39 | 338 |
| | | | | Wheat germ 8 g. | | | |
| | | | | 176 | Regular | 4 | — |
| | | | | Wheat germ 4 g. | | | |
| 921 | 132 | " | 282 | 160 | Regular | 20 | 368 |
| | | | | Wheat germ 6 g. | | | |
| 44 | 116 | " | 299 | 176 | Regular | 19 | 362 |

The first 4 birds had previously received caseinogen and the yeast concentrate with the results already described. Wheat germ extract in doses equivalent to 6 g. cleared up the cardiac irregularity in each case after an interval of 13-23 days. This curative interval is considerably longer than that usually observed with whole wheat, but it should be noted that the method of preparing the extract involves considerable losses, and the actual amount administered

is probably substantially less than the equivalent of 6 g. of dry wheat germ. It will be seen that in three of these birds there is a rise in weight which somewhat exceeds the initial weight maximum. The significance of this fact has already been discussed. The remaining birds received wheat germ extract immediately following the depletion period. Birds 921 and 940 received the equivalent of 4 g. of the extract, and in the case of the latter this dose failed to improve the cardiac condition in a period of 39 days. An increase in the dose to the equivalent of 8 g. resulted in complete recovery of the heart in 4 days.

A final experiment in which a group of birds, after depletion on polished rice, were returned to whole wheat, provides further evidence for the view that restoration of heart block can be effected independently of the state of nutrition of the bird as judged by its body weight. Table VII shows that restoration of the heart occurred within 2-5 days after commencement of a whole wheat diet, and at a time when recovery of body weight was still incomplete.

Table VII.

| Bird | Maximum weight g. | Weight after depletion on rice g. | Weight at time of recovery of heart g. | Heart rate after depletion on rice | Heart rate after recovery on wheat | Time for recovery of heart days |
|------|-------------------|-----------------------------------|--|------------------------------------|------------------------------------|---------------------------------|
| 142 | 488 | 317 | 400 | 100 Dropped beats | 156 Regular | 2 |
| 912 | 346 | 229 | 293 | 128 " | 240 " | 5 |
| 157 | 450 | 342 | 419 | 80 Regular | 200 " | 5 |
| 23 | 384 | 251 | 310 | 108 Dropped beats | 184 " | 3 |

SUMMARY.

The experiments outlined in this paper, provide evidence for the view that one factor limiting the restoration, after depletion, of full weight of pigeons on a diet of polished rice supplemented by a yeast concentrate is an inadequate allowance of protein in the diet. Where this partial deficiency is made good by the addition of caseinogen full restoration of weight usually occurs. It is not finally proved that caseinogen, purified in the manner described, provides no factor other than protein, and it would therefore be premature to draw conclusions from these experiments as to the necessity for vitamin B₃ in pigeon nutrition. The evidence does however indicate that maintenance of, or recovery to, full weight may be compatible with the persistence of a cardiac arrhythmia of dietary origin. This dietary constituent can be extracted from wheat germ, and its administration in amounts equivalent to 6-8 g. leads to complete restoration of function.

I desire to record my sincere thanks to Prof. Peters for his continued interest and advice.

REFERENCES.

- Aykroyd (1930). *J. Hygiene*, **30**, 357.
 Carter (1930). *Biochem. J.* **24**, 1811.
 ——— Kinnersley and Peters (1930). *Biochem. J.* **24**, 1832.
 Kinnersley and Peters (1927). *Biochem. J.* **21**, 778.
 O'Brien (1934). *Biochem. J.* **28**, 926.
 Osborne, Van Slyke, Leavenworth and Vinograd (1915). *J. Biol. Chem.* **22**, 259.
 Morris (1933). Dissertation.
 Sugiura and Benedict (1923). *J. Biol. Chem.* **55**, 33.
 Williams and Eddy (1931). Carnegie Report.