CCCXXVIII. MAINTENANCE NUTRITION IN THE PIGEON. THE INFLUENCE OF DIETARY PROTEIN AND VITAMIN B_3.

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(*Received November 1st, 1935.*)

The multiple deficiencies inherent in an exclusive diet of polished rice have for some time been recognised as being contributory to the syndromes of human beriberi and avian polyneuritis. In addition to a deficiency in certain vitamin factors in polished rice the adequacy of the protein intake on such a diet has been questioned by several workers who, when studying the vitamin requirements of birds, made use of synthetic diets with caseinogen as the source of protein [Sugiura and Benedict, 1923] and daily supplements of meat protein to a polished rice diet [Block *et al.*, 1932], or autoclaved whole wheat [Morris, 1933]. The effect of a deficiency of protein has been demonstrated by Carter [1934] who found that pigeons on a polished rice diet supplemented by a concentrate of vitamin B_1 and the fat-soluble vitamins failed to regain their maximum weight after a short period of depletion, whereas the addition of caseinogen sufficed to ensure full weight recovery. These observations did not completely agree with those of O'Brien [1934] who in the course of an investigation on the nature of vitamin B_3 found that a caseinogen supplement to such a diet promoted an initial gain in weight but failed to restore maximum weight. An extract of hydrolysed wheat germ containing vitamin B_3 and the hydrolytic products of wheat proteins always induced a gain in weight comparable with that seen in birds on a whole wheat diet. The experience of these workers suggests that at least two factors are playing a part in the partial weight restoration of pigeons on a diet of rice supplemented with vitamin B_1 concentrates; (*a*) a lack of protein and (*b*) vitamin B_3 deficiency. In this paper experiments are recorded which throw further light on this hypothesis.

It seemed to us possible that the severity of the initial depletion of the stored vitamins might determine the subsequent response to caseinogen. In those birds which retained much of their original store of vitamin B_3 as a result of a short period on polished rice the addition of vitamins B_1 and B_5 together with caseinogen should ensure a full recovery to maximum weight. On the other hand birds submitted to a more prolonged régime of polished rice involving severe loss of weight might be expected to be deprived of much of their stored vitamin B_3, and consequently would only regain their maximum weight if this factor, in addition to those mentioned, were also included in the diet. No precise information as to the period required to deplete the pigeon of vitamin B_3 is available although Carter *et al.* [1930] suggested that this might exceed 30–35 days. The experiments here described substantiate these views. It has been observed that birds on a polished rice diet supplemented with vitamin B_1 concentrates showed marked gains in weight when given protein in the form of caseinogen or gluten. These gains in weight are, however, appreciably influenced by the preliminary

degree of vitamin depletion. In this connection it may be mentioned that the extent of the initial loss in weight is a more reliable guide to the severity of the depletion than the duration of this depletion. In those cases where the degree of depletion is considerable a failure to reach maximum weight is seen but can be remedied by the administration of vitamin B_3 concentrates in the form of extracts of plant and animal tissues, particularly of liver and wheat germ.

Experimental.

Method.

The birds under test were caged singly under conditions which have been previously described. Their basal diet consisted of washed and autoclaved polished rice (95 %), McCollum's salt mixture (5 %) and a small amount of grit in the form of oyster shell. Every alternate day they were given cod-liver oil (10 drops). The source of vitamin B_1 was an alcoholic concentrate from activated norite [Kinnersley *et al.*, 1933] which contains, in addition to vitamin B_1, vitamin B_5 and vitamin B_6. Throughout this communication when speaking of vitamin B_1 concentrates we refer to this impure concentrate. The daily dose of this concentrate was equivalent to 10–12 pigeon day doses of vitamin B_1 (11·1 pigeon day doses equal approximately 1 I.U. [Kinnersley and Peters, 1935]). Caseinogen (Glaxo, alcohol-extracted) and wheat gluten (Harrington) were not further purified and were administered in dry form. When the birds received more than one supplement the different doses were given at intervals of not less than 2 hours.

Varying degrees of depletion of the stored vitamins were effected by allowing the birds to feed *ad libitum* on the basal diet for different periods before giving them additional supplements.

Effect of vitamin B_1 concentrate.

The typical response of pigeons receiving daily doses of the vitamin B_1 concentrate after a period of depletion on the basal diet is a rise in weight which, although rapid for a few days, reaches a plateau substantially below the maximum level for the bird. No further rise in weight is observed if the dose of the concentrate is increased to as much as 60 doses per day. This fact has now been well established. From a study of the influence of the duration of depletion on the weight response of the pigeon to vitamin B_1 concentrates alone some evidence has been obtained of the need of a further factor or factors.

The duration of the initial depletion appears to influence the subsequent response in weight change to the vitamin B_1 concentrate. 43 birds received daily 12 doses of the vitamin concentrate after an initial depletion period the duration of which varied from 14 to 34 days. In the short depletion group the body weight did not fall below 70 % of the maximum (Group 1), whereas in the long depletion group the weight declined below 70 % of the maximum (Group 2). The average gain in weight for birds of Group 1 is 57 g. as compared with 35 g. for those of Group 2. Further, if the vitamin concentrate is administered for a lengthy period the weight plateau is not always maintained but may be succeeded by a very slow decline, which in some cases may result in almost complete relapse to the original depletion weight (Fig. 1). In a series of 10 birds 12 doses of the concentrate were given daily for periods of 106–201 days. Whereas the average maximum gain of weight amounted to 76 g., the average nett gain over the whole period was only 52 g. In two cases, it was observed that the earlier rise in

weight was followed by a slow fall which actually resulted in a final nett loss. In other birds this decline is present though much less conspicuously, and one bird showed an exceptionally large initial rise with practically negligible decline later.

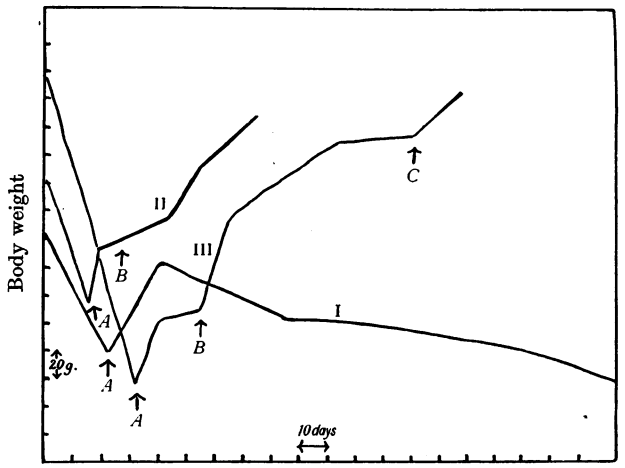

Fig. 1. Illustrates the response of birds on polished rice to various supplements. At *A*, 12 doses of vitamin B_1 concentrate; at *B*, 12 B_1 + 2 g. caseinogen; at *C*, 12 B_1 + 2 g. caseinogen + vitamin B_3 concentrate were administered.

Effect of vitamin B_1 concentrate supplemented with protein.

Determination of the daily rice intakes of pigeons fed on the basal diet supplemented with vitamin B_1 with and without added protein have been compared with the intakes of these birds on whole wheat. The data summarised in Table I show that polished rice as a basal diet satisfies the caloric requirements

Table I. *Calorie and protein intake on rice and whole wheat.*

No. of birds	Nature of diet	Av. daily food intake g.	Av. daily calorie intake Cals.	Av. daily protein intake g.	Days on test
8	Rice + vitamin B_1 concentrate	26·7	96·5	1·72	23–27
8	Rice + vitamin B_1 concentrate and gluten	21·1	85·6	3·37	42–50
6	Rice + vitamin B_1 concentrate and caseinogen	21·2	86·0	3·38	14–50
5	Whole wheat	30·8	105·9	3·36	21

under our conditions. It is seen that the consumption of polished rice by a bird suffices to give an intake of 85–95 Cals. per day; that of whole wheat 105 Cals. These figures agree satisfactorily with the values of Sugiura and Benedict [1923] of 70 Cals. per day, and of Block *et al.* [1932] of 52–93 Cals. per day for normal maintenance. In the present case the significance of the difference in the caloric intake of birds on rice and whole wheat is minimised by the fact that in an experiment with 6 birds in which supplements of liver fat and rice in doses equivalent to 12–35 Cals. were administered no increase in weight was observed with this supplement. On the other hand birds on the rice diet supplemented with the vitamin B_1 concentrate and 2 g. protein ingest 85 ± 10 Cals. per day and show

good increases in weight. The daily intake of protein is important. Polished rice [Plimmer, 1921; Rosenheim and Kajiura, 1908] contains 6–7 % protein whilst whole wheat which forms a satisfactory diet for the pigeon contains 11 %. From these figures and the values of the food intakes the daily consumption of 1·2–1·7 g. protein by a bird on polished rice is about 50 % lower than the 3·37 g. consumed by the bird on whole wheat. That the low percentage of protein in polished rice contributes considerably to the failure of birds to regain maximum weight on polished rice supplemented by vitamin B_1 concentrate is borne out by experiments designed to show the effect of including an additional protein supplement in the daily ration. In these experiments we have endeavoured to simulate a diet of whole wheat by raising the protein intake to 3·2–3·6 g. per day by supplements of caseinogen or wheat gluten. In all cases where the protein intake is increased there is a further rise in weight above the level reached with the vitamin concentrate alone. This response, however, does not result in full recovery in all cases but appears to be determined by the intensity of the original depletion of stored vitamins. The effect of a supplement has been studied in 70 birds. In general birds under test were submitted to a depletion period of varying duration, and thereafter each received daily 12 doses of the vitamin concentrate until the weight curve showed a definite plateau. A supplement of 2 g. caseinogen or 2 g. gluten was administered until maximum weight or a constant weight level had been attained. In a few cases the protein supplement was begun simultaneously with the administration of the vitamin concentrate. In the case of 6 birds administration of caseinogen was preceded by a short period during which they received certain amino-acid fractions obtained by hydrolysis of caseinogen. The data are summarised in Table II for caseinogen and gluten respectively. In the experiment with caseinogen it will be seen that for those birds the weight of which did not fall below 70 % of the maximum during the preliminary depletion period full recovery of weight occurred in 70 % of the cases. The time taken for full recovery in these birds varied from 30 to 60 days. On the other hand only 46 % of those birds submitted to a longer depletion subsequently recovered maximum weight with the caseinogen supplement. Among the birds of this group, which failed to make full recovery death occurred in 40 % of the cases. The efficiency of the caseinogen was in no way impaired by autoclaving it at 120° for 2·75 hours. Two birds of the long depletion group received daily 2 g. autoclaved caseinogen. Bird 643 regained maximum weight within the normal period whilst the weight of bird 822 was not further improved by the substitution of unheated caseinogen. The results with gluten were substantially the same as with caseinogen. In the short depletion group no less than 87 % regained maximum weight as compared with 44 % for the birds submitted to long depletion. Four birds of the short depletion group had been previously used in the corresponding test with caseinogen. The weight curves show a close parallelism for the two supplements. The fact that, when the protein intake is increased by administration of caseinogen or gluten, gains in weight are produced, suggests that the inadequacy of the protein intake of birds on polished rice constitutes one factor limiting the full weight recovery under the present experimental conditions. This confirms the conclusion reached from the study of the food intakes on whole wheat and polished rice (Table I).

The failure on the part of some birds, submitted to a long period of initial depletion, to respond fully to the protein supplements is open to several possible interpretations. Prolonged subjection to a deficient diet may lead to irreversible tissue destruction which is not reparable even when a complete diet is substituted. This hypothesis is only tenable if a bird which fails to regain weight on the basal

Table II. *Influence of depletion on the response of pigeons to caseinogen and gluten.*

Short depletion						Long depletion				
Bird	Max. wt. g.	Wt. at end of depletion period g.	Depletion wt. expressed as % of max. wt.	Final wt. g.		Bird	Max. wt. g.	Wt. at end of depletion period g.	Depletion wt. expressed as % of max. wt.	Final wt. g.
					Caseinogen.					
721	338	249	73	358		142	420	276	65	419[3]
719	393	299	76	398		691	432	299	69	419[1]
186	370	288	77	370		297	392	254	64	386
689	437	335	76	447		296	360	220	61	368
733	422	331	78	470		518	340	222	65	340
683	397	296	74	393		914	324	207	64	319
426	380	303	79	405		528	392	252	64	406
63	385	296	77	396[3]		692	406	269	66	394
735	387	314	81	394[1]		157	450	307	68	450
418	357	252	70	369		248	500	330	66	495
879	398	297	74	397[3]		613	332	212	64	338
736	340	243	71	350		833	328	216	66	323
157	450	337	75	437		643	284	192	67	281[4]
72	410	294	71	410[1]		579	495	272	55	455
722	443	318	71	392		445	426	261	61	408
289	440	330	75	388[3]		17	440	234	53	355[2]
190	500	377	75	440[3]		881	386	222	57	286[2]
299	400	280	70	369[1]		398	307	192	62	274[2]
247	428	314	73	391[1]		761	302	192	63	231[2]
287	474	345	72	433[1]		174	352	196	55	214[2]
						66	368	220	60	271[2]
						9	410	264	64	358
						293	400	224	56	369
						291	400	264	66	385
						91	355	201	56	345
						633	348	226	65	268[2]
						822	425	276	65	407[4]
						294	374	220	58	327
					Gluten.					
721	390	292	77	434		733	500	340	68	496
719	398	286	72	419		809	358	247	69	378
157	460	342	76	450		836	418	290	69	433
357	380	294	77	379		438	390	265	68	430
692	410	296	72	406		811	428	278	65	430
186	392	293	76	386		882	465	315	67	460
881	386	272	70	362		541	436	303	69	427
299	469	357	76	402[5]		689	500	340	68	458
						396	364	248	68	282[5]
						125	359	212	59	253[6, 2]
						912	360	246	68	314[5]
						829	338	198	58	222[2]
						785	364	194	53	187[2]
						831	352	230	65	223[2]
						802	348	202	59	298
						21	459	302	65	357[6]

[1] Amino-acid fractions of caseinogen given prior to caseinogen.
[2] Died during course of experiment.
[3] Prolonged period on vitamin B_1 concentrate prior to caseinogen.
[4] Autoclaved caseinogen given prior to caseinogen.
[5] Gliadin supplement given prior to administration of gluten.
[6] Glutenin supplement given prior to administration of gluten.

diet fails also when placed on a whole wheat or mixed corn diet. It is also possible that unpalatability of the basal rice diet may produce a restriction in the food intake with the consequent failure to attain maximum weight. Although some reduction of the caloric intake has been observed in birds on rice as com-

pared with those on whole wheat, it seems unlikely that mere unpalatability of the diet will account for the incomplete weight recovery, since the majority of the short depletion birds are fully restored to maximum weight and in the case of the females, the diet meets the requirements of egg laying. There remains the possibility that prolonged depletion has produced a deficiency of a factor which we here call vitamin B_3. If this is the case, birds which have failed to regain maximum weight when supplied with vitamin B_1 and protein, should do so when vitamin B_3 is administered.

The effect of vitamin B_1, vitamin B_3 and protein.

Three types of extract have been used in these experiments. Preparation A was obtained as follows: 2 kg. of sheep-liver fresh from the slaughter house were finely minced and treated overnight with 8 l. 97 % alcohol. After filtering the clear yellow solution the liver residue was again twice extracted with alcohol and the combined filtrates concentrated *in vacuo* at 40–50° to a small volume. During this process a considerable amount of lipoid material separated out. The extract was now shaken repeatedly with ether until the ether remained colourless, and a white solid separating during this treatment was filtered off. The syrupy yellow fluorescent liquid (preparation A) was administered in amounts equivalent to 5–10 g. of original wet liver. The ethereal solution of fats was freed from ether and taken up on polished rice (preparation B). It was administered in doses of 0·5–1·5 g. The third preparation C was obtained by submitting dry powdered liver, previously extracted with 97 % alcohol, to mild hydrolysis with $N/10$ HCl for 90 min. The hydrolysate was filtered hot and after neutralisation was given in doses equivalent to 5–10 g. dry powder. It was prepared fresh daily to prevent loss of vitamin B_3 potency [O'Brien, 1934]. Table III summarises data which illustrate the vitamin B_3 activity of these preparations. The birds used in the

Table III. *Influence of liver preparations.*

Bird	Max. wt. g.	Nature of supplement	Wt. before and after administration of liver prep. g.		Duration of liver supplement days	Gain in wt. g.	Av. gain in wt. per day g.
722	442	Caseinogen 2g. + alcoholic extract of liver	371	420	8	49	6·1
9	410	,, ,,	335	389	7	54	7·7
722	442	Caseinogen 2g. + preparation A	411	445	30	34	1·1
9	457	,, ,,	382	442	35	60	1·7
579	495	,, ,,	449	478	13	29	2·2
147	475	,, ,,	329	426	41	97	2·3
541	436	,, ,,	408	452	11	44	4·0
802	348	Gluten 2 g. + preparation A	298	315	14	17	1·2[1]
579	495	Caseinogen 2g. + preparation C (≡5g. dry liver)	449	478	13	29	2·2
147	475	,, ,,	427	486	21	59	2·8
147	475	,, ,,	434	464	12	30	2·5
722	442	,, ,,	434	460	4	26	6·5
722	442	Caseinogen 2g., rice 2g. + preparation B 0·5g.	369	371	19	2	Nil
9	410	,, ,,	340	335	19	− 5	Nil
722	442	Caseinogen 2g., rice 2g. + preparation B 1·5g.	420	411	32	− 9	Nil
823	334	,, ,,	222	217	17	− 5	Nil[2]
883	384	,, ,,	286	280	13	− 6	Nil[2]
809	358	,, ,,	261	262	17	1	Nil
812	344	,, ,,	238	247	6	9	1·5[2]
880	400	,, ,,	264	248	5	−16	Nil[2]

[1] Subsequently regained maximum weight without liver supplement.
[2] Died during course of experiment.
NOTE. All birds received basal rice diet and 12 doses vitamin B_1 daily. One bird failed to respond to preparation A or subsequently to wheat.

tests had maintained a stationary weight for 20–60 days before the administration of the preparations.

In a preliminary test birds 722 and 9 were given doses of a crude alcoholic extract of liver. During the course of this test, which was not continued until maximum weight had been established, the high daily average gain in weight of 6–8 g. was observed. Tests performed with the ether-soluble fraction, preparation B, and the aqueous fraction, preparation A, showed clearly that the activity was concentrated in the latter. Thus preparation A gave daily gains in weight of 1–4 g. during the period of testing for 6 birds. Bird 445 which failed to rise in weight was an exception and did not respond subsequently to whole wheat. In 3 birds tested, the experiment was continued until maximum weight had been established. Preparation C also showed considerable activity in spite of preliminary extraction of the fresh liver with alcohol. The observed increments in weight though small are in our experience significant particularly since the growth rate is slow when a bird approaches its maximum weight. It was hoped that a more potent preparation might be obtained from autolysed liver. We have found, however, little or no activity in preparations of this type. The extracts of vitamin B_3 used in these experiments contained amounts of the antineuritic factor which would increase the dose of the vitamin B_1 given daily by 7–14 units. Our experience of the effect of the daily administration of 20–30 doses of vitamin B_1 leads us to conclude that the activity of our liver preparations cannot be explained in terms of their vitamin B_1 content but is due to the presence of vitamin B_3. It is to be noted that the vitamin B_3 effect of these concentrates fully manifests itself only when the birds' intake of protein is adequate. For in an experiment with 4 birds on polished rice supplemented with vitamin B_1, doses of liver extract equivalent to 20 g. liver produced rises in weight of 0·5–1·5 g./day which are distinctly lower than the initial daily increments observed in birds of short depletion on polished rice when caseinogen is added.

The storage of vitamin B_3.

The evidence presented suggests that significant depletion of vitamin B_3 only becomes manifest when the body weight has fallen below 70 % of the maximum. In most birds this stage is reached within 25–35 days, *i.e.* after a period rather longer than is required to deplete the reserves of the antineuritic vitamin. The depletion on polished rice is not complete since in all cases studied some weight recovery is made when protein is added to the diet. Nevertheless, the rate of depletion of vitamin B_3 may be influenced by the multiple deficiencies of polished rice. Thus, a different picture is obtained if the birds, originally at maximum weight, are placed on a diet complete in all known dietary constituents except vitamin B_3. Such a condition is approximately attained if the bird at maximum weight is transferred from wheat to our basal diet supplemented with vitamin B_1 and protein. 18 birds have been maintained under these conditions for periods of 52–146 days. In some of these cases the bird had previously risen to maximum weight without addition of the liver supplement; in others this had been given to establish maximum weight. Weight at this level without additional vitamin B_3 is not fully maintained (Table IV). The decline which occurs, however, is very gradual and is of a different order from that seen in birds on polished rice alone. In certain cases a vitamin B_3 preparation was given at the end of the maintenance period. Recovery to maximum weight then occurred (Table V). It would appear that the reserves of vitamin B_3 in the bird are held in some firmly bound state and are only liberated as the result of tissue disintegration such as occurs on a diet involving multiple deficiencies.

Table IV. *The decline in weight of birds on a diet of rice, vitamin B_1 and protein.*

Typical cases from a group of 18 birds.

Bird	Nature of previous diet	Maximum wt. g.	Final wt. g.	Loss in wt. g.	Duration of maintenance days
722	Rice + vitamins B_1 and B_3 and caseinogen	460	424	36	72
147	Rice + vitamins B_1 and B_3 and caseinogen	454	434	20	70
142	Rice + vitamin B_1 and caseinogen	420	404	16	58
157	Rice + vitamins B_1 and B_3 and gluten	446	427	19	76
733	Rice + vitamin B_1 and gluten	497	455	42	91
186	Rice + vitamin B_1 and gluten	386	369	17	53

Table V.

Bird	Wt. at end of maintenance period g.	Wt. after vitamin B_3 addition g.	Gain in wt. g.	Duration of vitamin B_3 addition days
248	475	489	14	13
296	345	363	18	4
186	369	386	17	9
291	385	382	−3	9
733	441	484	43	52
157	424	450	26	22

Discussion.

The experiments described indicate that the daily consumption of rice by a pigeon does not suffice to ensure an intake of protein which is adequate to restore and maintain maximum weight. This conclusion is subject to certain qualifications. In the first place, it has been assumed that the supplements of caseinogen and gluten carry no significant amount of any essential dietary constituent other than protein. The necessity for additional protein is shown by the fact that birds after depletion on polished rice show initial gains in weight of 0·5–1·5 g./day when given liver extract alone as compared with initial gains in weight of 5–10 g. when receiving caseinogen in addition. Secondly, while we believe that our experiments allow us to conclude that limitation of protein rather than total caloric intake is one of the factors which prevent full weight recovery on polished rice, the evidence does not differentiate between limitation of protein *per se* and a deficiency of some specific amino-acid. Experiments bearing on this point are still in progress. Thirdly, the response to an adequately adjusted protein intake is only partial or may be altogether absent if the preliminary period of depletion on polished rice results in a fall of weight below 70 % of the maximum. In such cases recovery is limited by the appearance of a deficiency of vitamin B_3 which is wholly or largely lacking in the diet. This deficiency can be corrected by the administration of vitamin B_3 in the form of extracts of liver. Our experiments, therefore, seem to indicate that two factors at least are necessary to promote weight restoration in pigeons on polished rice supplemented with vitamin B_1: (*a*) protein in adequate amount and (*b*) vitamin B_3.

Summary.

1. Pigeons on a polished rice diet supplemented with vitamin B_1 concentrates alone show an early rise in weight which may be followed by a slight decline suggesting deprivation of a stored factor or factors.

2. Comparison of the food intakes of birds on such a diet and on whole wheat shows that although the caloric intake of the bird is satisfactory for its normal maintenance, the amount of protein ingested is inadequate.

3. It is shown that this deficiency of protein in polished rice can be remedied by the addition of caseinogen or gluten to the basal diet whereby a recovery in weight is produced.

4. The extent of this recovery varies with the degree of depletion of a stored vitamin factor, namely vitamin B_3. 54 % of the birds subjected to long depletion periods fail to regain their original maximum weight when receiving a vitamin B_1 concentrate and protein alone.

5. This failure to rise in weight is remedied by the addition of vitamin B_3 in the form of liver concentrates to such diets.

The authors wish to thank Prof. Peters for his continued encouragement and advice. One of us is indebted to the Medical Research Council for a personal grant.

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