CCXXIV. MAINTENANCE NUTRITION IN THE PIGEON. FURTHER EVIDENCE FOR THE PRESENCE OF DIETARY ESSENTIALS IN YEAST AND LIVER AND THEIR RELATION TO VITAMIN B₆

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(Received 11 September 1939)

EVIDENCE in support of the view that at least four components of the vitamin B complex are essential in the normal nutrition of the pigeon has already been reported briefly by us [Carter & O'Brien, 1937, 1, 2]. By treatment of a yeast concentrate with fuller's earth we have obtained an adsorbed fraction (yeast fuller's earth eluate fraction) which exhibits a supplementary relationship in the nutrition of the pigeon with the material remaining in the filtrate after this procedure. Administration of these fractions together to birds already receiving supplements of aneurin and riboflavin resulted in restoration to maximum weight. A similar relationship has been found to exist between our yeast eluate fraction and the filtrate obtained after treatment of a liver extract with fuller's earth. We think it desirable to present in greater detail the evidence for these findings. By the use of methods which differ somewhat from our own, Edgar *et al.* [1938] have obtained fuller's earth eluate fractions of liver and yeast which supplement the activities present in the corresponding fuller's earth filtrates in relation to rat nutrition. With regard to vitamin B₆ they make the important statement "Since the crystalline vitamin completely replaces our eluate fractions in the diet of the rat, it seems probable that vitamin B₆ is the only essential nutrient for the rat present in our yeast and liver eluates. The possibility, however, still exists that other essential nutrients may be common contaminants of our eluate fraction. . . ." Our own preliminary experience of the contribution of vitamin B₆ to the activity of our eluate fractions in regard to the pigeon is reported in the present paper.

Methods

Birds were maintained singly in cages under conditions which have been previously described. They received a basal purified diet [Carter & O'Brien, 1937, 1] and, in addition to other supplements, cod liver oil at regular intervals. Crystalline aneurin, a synthetic i.g. product, has been used in all experiments. Riboflavin was prepared from ox liver using frankonite and lead sulphide as adsorbents. The fuller's earth eluate and filtrate fractions were prepared as follows.

A crude extract of yeast, partially purified by preliminary treatment with lead acetate followed by baryta, was used as starting material. This extract, which corresponded to 100 kg. yeast, was diluted to 100 l. with water, acidified to pH 1 with H₂SO₄ and treated with 400 g. fuller's earth. After stirring for several hours the extract was filtered on Büchner funnels, the pH of the filtrate

was adjusted to 1.0 and three further treatments with fuller's earth were carried through. The fuller's earths were washed separately and then extracted 4-5 times with a mixture of pyridine, methyl alcohol and water (1 : 1 : 4). The combined eluates were concentrated *in vacuo* to remove pyridine and methyl alcohol, the residue was taken up in a small volume of water and 4 vol. of ethyl alcohol were added. The clear filtrate obtained after the removal of a large gummy precipitate was concentrated to remove alcohol, and, after adjusting the pH to 1, was thoroughly shaken four times with 3 vol. amyl alcohol to remove traces of filtrate factor. The aqueous fraction was then separated, traces of amyl alcohol were removed and the volume was adjusted so that 1 ml. corresponded to 1 kg. yeast. This fraction will be described as the yeast fuller's earth eluate fraction. Attempts were made to obtain a preparation of the filtrate factor from the filtrate of the yeast extract after its treatment with fuller's earth by adsorption on charcoal at pH 1 and 7; they were unsuccessful. An active preparation of the yeast filtrate factor was obtained by submitting Peter's eluate from yeast to successive fuller's earth treatments. The filtrate was concentrated and alcohol added to 50 % concentration in order to remove excess of inorganic constituents. A similar fractionation has been carried out on alcoholic extracts of ox liver after the preliminary removal of ether-soluble material and phosphatides. It has been found possible to remove all but the smallest traces of riboflavin by frankonite treatment at pH 7. Thereafter successive treatments with fuller's earth at pH 1 as described above were employed. In this way a liver fuller's earth eluate fraction and a corresponding filtrate (vitamin B₃) were obtained.

The activity of the above fractions has been tested on pigeons previously depleted on the basal diet alone to a weight level of about 70 % of the maximum and which have thereafter made a partial recovery to a steady submaximal plateau following daily additions of 40 μ g. aneurin and 80 μ g. riboflavin. The duration of the initial period is of cardinal importance in the demonstration of the need for eluate and filtrate factors by the pigeon, since these factors are stored much more tenaciously than is aneurin. After short periods of depletion supplements of aneurin alone may ensure complete weight recovery in a considerable proportion of cases. Only birds whose recovery with aneurin and riboflavin was incomplete were employed in testing the activities of the eluate and filtrate fractions.

RESULTS

Effects of fuller's earth eluates of yeast and liver

Table I shows the response obtained with daily supplements of yeast fuller's earth eluate. The selected group of results is typical of those which have been

Table I

Bird	Max. wt. g.	Init. wt. g.	Final wt. g.	Gain in wt. g.	Days	Gain in wt. per day g.
17	436	377	436	59	4	15
7	422	373	424	51	6	8.5
780	428	374	445	71	7	10
568	420	382	430	48	6	8
583	320	271	329	58	9	6.4
28	382	330	343	13	5	2.6
533	399	290	304	14	8	1.7
846	436	361	378	17	6	2.8
264	412	278	321	43	13	3.3
700	452	334	416	82	10	8.2 Subsequent decline on continuing eluate

observed in 25 birds. In about 25 % of these the ensuing weight rise is maximal while in the remainder a definite but incomplete response is seen. The results are comparable with those reported for vitamin B₆ concentrates [Carter & O'Brien, 1937, 2]. We have been at pains to exclude possible contamination with traces of factors which are present in the filtrate fraction. Thorough washing of the fuller's earth after adsorption and exhaustive extraction with amyl alcohol of the eluate presumably ensures this, but we have had in mind the possibility that rice starch in the basal diet may carry traces of the filtrate factors. We are at present engaged in further work to test this point. Table II shows similar effects for the liver fuller's earth eluate fraction.

Table II

Bird	Max. wt. g.	Init. wt. g.	Final wt. g.	Gain in wt. g.	Days	Gain in wt. per day g.
782	380	305	365	60	8	7.5
8	400	314	384	70	32	2.2
568	422	374	411	37	14	2.6
814	500	314	378	64	12	5.3
263	380	247	339	92	7	13
20	500	409	442	33	10	3.3
536	432	326	385	59	9	6.5
3	462	311	356	45	7	6.4
777	500	422	456	34	8	4.2
468	500	368	417	49	17	2.9

With this preparation also restoration to maximum weight occurs in a certain proportion of cases (17 %) and in the remainder the response is incomplete.

Effects of fuller's earth filtrates of yeast and liver

The effects of administration of fuller's earth filtrate from yeast and liver are summarized in Table III.

Table III

Bird	Max. wt. g.	Init. wt. g.	Final wt. g.	Gain in wt. g.	Days	Gain in wt. per day g.
Effect of yeast filtrate						
531	491	430	454	24	11	2.1
605	500	383	389	6	5	1.2
661	420	320	345	25	8	3.1
103	380	270	271	1	15	Nil
899	412	357	366	9	5	1.8
Effect of liver filtrate						
44	500	386	408	22	7	3.1
14	458	405	445	40	10	4
4	460	374	384	10	15	0.7
899	412	357	366	9	5	1.8
881	500	357	358	1	7	Nil

In the absence of the eluate fraction our earlier preparations of yeast filtrate sometimes restored maximum weight. This may have been due to incomplete removal of factors adsorbable on fuller's earth, since later preparations of yeast or liver filtrates which had been submitted to at least four successive treatments with fuller's earth gave an almost negligible response.

Effects of fuller's earth eluates and filtrates of liver and yeast

The slight response to the filtrate fractions administered alone is not to be taken as evidence of their inactivity for the pigeon. In cases where birds had failed to regain maximum weight after receiving the yeast eluate fraction a further supplement of either yeast or liver filtrate resulted in complete restoration of weight. Some typical results are summarized in Table IV.

Table IV

Bird	Max. wt. g.	Init. wt. g.	Final wt. g.	Gain in wt. g.	Days	Gain in wt. per day g.
Effect of liver filtrate after yeast eluate						
28	382	343	377	34	14	2.3
595	364	264	360	96	15	6.4
535	393	290	366	76	14	5.3
263	400	331	383	52	8	6.5
416	452	321	415	94	26	3.6
Effect of yeast filtrate after yeast eluate						
21	420	383	404	21	9	2.3
899	412	366	390	24	16	1.5
780	428	391	410	19	13	1.4
Effect of liver filtrate after vitamin B ₆						
1	415	339	386	45	10	4.5
12	460	347	389	42	17	2.5
17	436	399	432	34	6	5.6

It is noteworthy that the response to liver filtrate in three birds which had previously received 40 μ g. crystalline vitamin B₆ daily is comparable with that seen when yeast eluate had preceded administration of the filtrate. The average gain in weight (40 g.) is about three times greater than the average gain in weight (14.5 g.) of a group of six birds which received the same preparation of liver filtrate alone.

A similar restoration to maximum weight is observed in the great majority of birds which received yeast eluate after a preliminary response to yeast or liver filtrate. This is illustrated in Table V.

Table V

Bird	Max. wt. g.	Init. wt. g.	Final wt. g.	Gain in wt. g.	Days	Gain in wt. per day g.
Effect of yeast eluate after liver filtrate						
44	500	405	500	95	12	7.9
881	500	358	491	133	18	7.4
446	360	295	359	64	9	7.1
899	412	321	407	86	8	10.7
12	460	389	453	64	10	6.5
Effect of yeast eluate after yeast filtrate						
447	334	309	355	46	3	15.3
641	460	428	456	28	7	4
531	491	441	490	49	5	9.8
624	412	369	406	37	12	—
605	500	389	468	79	20	3.9

Effect of vitamin B₆

It is interesting to compare the response to crystalline vitamin B₆, now to be described, with the results already recorded for the fuller's earth eluate. The influence of vitamin B₆ in daily doses of 40 μ g. as a supplement to aneurin and riboflavin is shown in Table VI. It has only been possible to observe the behaviour of three birds owing to lack of material, and any conclusion must necessarily be provisional. Nevertheless, both from these results and from those in which vitamin B₆ followed administration of liver filtrate, we have gained the impression that the response to the crystalline product is less marked than that to the yeast fuller's earth eluate fraction. Table VI also illustrates the response to vitamin B₆ following administration of liver filtrate. The result leaves little room for doubt that vitamin B₆ is an essential nutrient for the pigeon. In this series of seven birds tested, all showed a definite response, though in only two can it be said that full weight restoration was obtained. It is possible that nicotinic acid may have been a limiting factor, since, in agreement with Harris [1939], we have found that increases in weight follow administration of large doses of this substance. Our findings in this connexion will be reported later.

Table VI

Bird	Max. wt. g.	Init. wt. g.	Final wt. g.	Gain in wt. g.	Days	Gain in wt. per day g.
Effect of vitamin B ₆						
1	415	339	339	0	10	Nil
12	460	312	347	35	11	3.1
17	436	369	399	30	12	2.5
Effect of vitamin B ₆ following liver filtrate						
7	422	344	420	76	11	7
122	370	316	367	51	7	7.3
13	390	318	350	32	11	2.9
4	460	334	384	50	11	4.5
11	490	440	455	15	9	1.6
173	440	411	422	11	4	2.7
180	427	365	379	14	4	3.5
Effect of vitamin B ₆ following nicotinic acid and liver filtrate						
5	345	271	343	72	7	10.3
523	459	389	455	66	5	13.2
7	500	471	493	22	3	7.3
3	376	358	366	8	4	2.0

In four birds which received vitamin B₆ following 5 mg. nicotinic acid and liver filtrate daily there was a marked response and almost complete restoration of weight in each case.

DISCUSSION

The data reported in this paper support the conclusion which we drew from results previously published [Carter & O'Brien, 1937, 2] that the pigeon requires at least two aqueous soluble factors in addition to aneurin and riboflavin. These additional factors are present in a fuller's earth eluate fraction (vitamin B₆ concentrate) and a fuller's earth filtrate fraction (vitamin B₃ concentrate) respectively. We have shown in unpublished experiments that our fuller's earth eluate fraction contains vitamin B₆, as indicated by its curative action upon rat dermatitis, whereas the filtrate fraction has no such effect. The supplementary action of vitamin B₆ in pigeon nutrition upon the effect of the filtrate factor compared with a similar action of the yeast eluate fraction indicates that vitamin B₆ contributes to the activity of vitamin B₃ concentrates. That it does not

account for the whole activity of the eluate fraction is suggested by our present evidence. This evidence indicates that the effect of vitamin B₆ is not so great as that of the yeast eluate fraction. Two possible reasons may account for this: the administration of inadequate doses of vitamin B₆, or the presence of one or more other active substances in the yeast eluate concentrate. The absence of appreciable further improvement in weight following increase in the dose of vitamin B₆ to 80 µg. seems to exclude inadequate intake of this substance. Alternatively, the presence of an additional factor in the eluate fraction must be considered. In this connexion it may be noted that vitamin B₆ is more effective in restoring maximum weight when nicotinic acid is administered than when it is not. While more evidence would be required to prove that it is this substance which, together with vitamin B₆, accounts for the full activity of the yeast eluate concentrate, attention may be drawn to the observation of Macrae & Edgar [1937] that nicotinic acid is present in eluate fractions similar to our own, while Harris [1939] has recently reported that nicotinic acid is required by the pigeon.

The weight-restorative action of our most recent preparations of the liver filtrate factor when given without the eluate fraction is negligible in comparison with that of the earlier preparations which had received less exhaustive treatment with fuller's earth. It is possible that the difference is due to the removal of nicotinic acid by this procedure. Such a liver filtrate fraction, however, shows marked activity when supplemented by the eluate fraction or by vitamin B₆. Further experiments are now in progress to substantiate these points.

SUMMARY

1. Evidence is presented to show that the pigeon requires, in addition to aneurin and riboflavin, a fuller's earth eluate factor and a fuller's earth filtrate factor. Both of these are present in yeast and liver. Methods are described for the preparation of concentrates of these factors.

2. The fuller's earth eluate fraction from yeast has a marked weight-restorative action, and when supplemented by the filtrate fraction weight recovery is maximal.

3. Crystalline vitamin B₆ in daily doses of 40 µg. also exhibits a supplementary relationship with the filtrate fraction. The activity of vitamin B₆ is less than that of the yeast eluate fraction. The possible reasons for this discrepancy are discussed.

4. The fuller's earth filtrate fraction of yeast or liver has little weight-restorative action when supplemented by aneurin and riboflavin, but its activity can be demonstrated when the yeast eluate fraction or vitamin B₆ is given in addition.

We wish to record our grateful thanks to Dr T. F. Macrae, Dr Lepkovsky and Merck and Co., Inc., U.S.A. for generous gifts of crystalline vitamin B₆, and to the Agricultural Research Council for a grant towards the expenses of this research. We thank Prof. R. A. Peters for his continued interest and advice.

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