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Vitamins Required by Pigeons

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ABSTRACT

Pigeons subjected to long depletion periods with thiamin as the only vitamin supplement become anemic and lose weight. The anemia and loss in weight are both due to multiple deficiencies. A fuller's earth adsorbate of tikitiki, a fuller's earth adsorbate of a liver extract, and a charcoal adsorbate of the liver extract filtrate, together supply all the water-soluble vitamins required by mature pigeons. A combination of any two was incomplete. Of the recognized water-soluble vitamins, thiamin, riboflavin, pyridoxine, and pantothenic acid are certainly required by the pigeon. The data indicate that nicotinic acid is also required, but are not regarded as conclusive. No evidence at all was obtained that the pigeon requires choline or inositol.

Vitamins Required by Pigeons

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Hogan, Richardson, Johnson, and Nisbet ('40) maintained pigeons for several weeks on simplified diets that were grossly deficient in water-soluble vitamins, and observed that they became anemic. Some of the erythrocytes of the anemic pigeons were abnormal, with some resemblance to the red corpuscles of sickle-cell anemia. The pigeons simultaneously suffered severe losses in weight, but it was concluded that the anemia was not due solely to inanition. Damoshek and Myerson ('40) confirmed our report in all essential details though they did not mention observing abnormal erythrocytes. This type of anemia was not reduced by riboflavin, nicotinic acid, or by wheat germ oil. Tikitiki healed the anemia promptly but seldom, or never, restored the birds to normal weight. A number of crude vitamin carriers or concentrates were prepared, but none was a reliable source of all the vitamins required to restore these pigeons to a normal condition.

In the continuation of the work now to be described, the object was to find a combination of vitamin concentrates that would heal the anemia and restore normal weight, and offer promise of usefulness in attempts at further concentration. A parallel step in attaining this objective was the identification of known vitamins that are required by the pigeon.

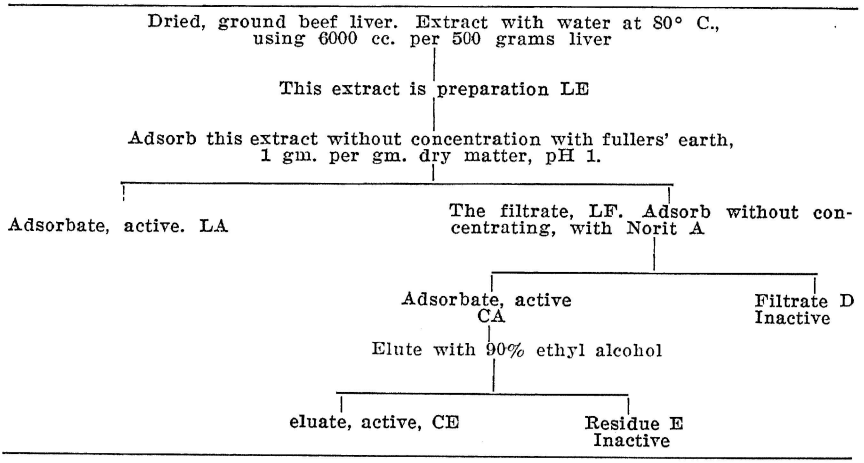
EXPERIMENTAL

An earlier publication ('40) should be consulted for details of the experimental procedure. The experimental ration No. 1669 is composed of casein 20, sucrose 71, salt mixture (Osborne and Mendel, '19) 4, cellulose 3, and cod liver oil 2 per cent. When the birds had developed polyneuritis or had lost approximately 30 per cent in body weight, 50 mcg. of thiamin were supplied daily per pigeon. The initial depletion period was usually 3 or 4 weeks. As a rule the hemoglobin level of each pigeon was determined each week thereafter by the method of Schultze and Elvehjem ('34), on blood drawn by puncture from a wing vein. In some instances, however, the change in weight was regarded as a sufficient index of nutritional state. As a rule the feeding of supplements was begun when the hemoglobin level had dropped, on the Newcomer scale, to about 60 per cent of normal. The necessity of long-continued and severe depletion periods is admirably demonstrated by Carter and O'Brien ('37). Periods that are too short will not deplete pigeons of some of the vitamins that are essen-

tial for their well-being, and thus give the impression they are not required at all. Typical responses during the depletion periods and subsequent stages are shown in Fig. 1.

All supplements were packed in gelatin capsules and hand fed daily. The supplements included: Tikitiki, prepared by the method of Wells ('21). A fuller's earth adsorbate of a rice bran extract, TA, prepared by the method of Williams, Waterman, and Keresztesy ('34). The other crude vitamin carriers were liver extract fractions, and are described in tabular form.

TABLE 1.—LIVER EXTRACT FRACTIONS.



LA should contain pyridoxine and riboflavin. CA and CE should contain pantothenic acid.

Dosage of supplements

B_1, thiamin, 50 mg. B_6, pyridoxine 15-20 mcg.
F, riboflavin, 40 mg. PA, pantothenic acid 280 mcg.
NA, nicotinic acid 1-10 mg.

TT, tikitiki	500 mg.
TA, fullers' earth absorbate of tikitiki	600 mg.
LE, liver extract	136-272 mg.
LA, fullers' earth adsorbate of liver extract	600 mg.
LF, filtrate from LA	200 mg.
CA, charcoal adsorbate of LF	300 mg.
CE, eluate of charcoal adsorbate	45 mg.

In order to avoid unnecessary confusion in presenting our data, it is necessary to mention in the beginning a fact that did not become evident for some time after our studies were underway. This is that anemia, as well as the weight loss, is the result of a multiple deficiency. It would happen frequently that a vitamin supplement, or com-

bination of supplements, would heal the anemia and restore normal weight, and then fail in a subsequent trial. It was supposed at first that at least one essential vitamin is labile, and that the inconsistencies were due to its destruction, but it developed later that this was erroneous. In the meantime a wide variety of crude vitamin carriers was tried in an attempt to find one that consistently restored the pigeons to a normal condition.

Crude Carriers that Supply the Water-Soluble Vitamins Required by Pigeons

As mentioned previously, tikitiki had proven to be very effective in healing the anemia, but had been somewhat lacking in the factors that are essential for weight maintenance. This preparation, therefore, was chosen for an attempt at further concentration, and a fuller's earth adsorbate was prepared, TA. Previous studies had shown that the adsorbate contains very little riboflavin, so the vitamin was added to the fuller's earth concentrate. As shown in Table 2, this combination lacked something of being entirely effective. Four pigeons recovered promptly from the anemia and finally attained 92 per cent of normal weight. The response of the others was indifferent.

It seemed necessary to look elsewhere for concentrates that would supply the missing vitamins, and as a water extract of liver had been useful with other animals it was decided to try it with pigeons. This extract, LE, was supplied to three pigeons. There were slight gains in weight and some increase in the hemoglobin content of the blood, but when used alone it was a failure.

It was then decided to combine the tikitiki adsorbate, TA, with the liver extract LE, and the two pigeons which received this combination recovered from anemia and reached normal weight. It was then decided to attempt further concentration of the liver extract, and it also was treated with fuller's earth. The adsorbate is LA, the filtrate LF. It seemed possible that the entire activity of the liver extract was segregated in one of these fractions, therefore each was tested separately on pigeons. Both elicited some response, but it was not evident at the time whether the original activity had been separated into two components, or whether it had merely been somewhat equally divided. For the purpose of further study it seemed desirable to attempt further concentration of the filtrate. A charcoal adsorbate, CA, was prepared from Extract LF which apparently was as effective as the original material. The eluate, LE, was equally active.

In order to determine whether the activity of Extract LE had been separated into two components, the adsorbate of tikitiki, TA, was combined separately with LA, and with LF, or with one of the deriva-

tives of LF. In some instances the combinations seemed completely effective, in others they were partly effective, and in still others they were almost completely inactive.

The presence of synthetic vitamins along with the crude carriers may call for some explanation. These pure compounds were included in the mixture of supplements in an attempt to determine the nature of their deficiencies. The observations mentioned up to this point are summarized in Table 2.

Some comment on Table 2 may be helpful in avoiding confusion as to its meaning, and the first line will be used as an example. The data are the average of 4 pigeons. The depletion period begins with the administration of thiamin, after the pigeons had developed polyneuritis, or had lost approximately 30 per cent of body weight. The supplements listed are administered when the pigeons have become definitely anemic or when it seems necessary in order to keep them alive, and this marks the beginning of the experimental period. In this example the pigeons regained a normal hemoglobin level and 92 per cent of normal weight, in an experimental period of 28 days. The experimental periods ended when recovery was complete, or when it became evident that increases in either weight or hemoglobin percentage had been arrested at a plateau below the normal level. The supplement of the pigeons described in the second line was the same as for those in the first, but they made only slight gains in weight, and the hemoglobin percentage declined rapidly. As will be noted in the tables these disturbing discrepancies were encountered frequently, and they made progress very slow. In time it became evident that they were due to variability in the degree of depletion of the pigeons and not to faults in the preparations. It should be pointed out that in many instances, in Tables 3 and 4 as well as in Table 2, the supplements listed were not administered simultaneously at the beginning of the experimental period. We were searching for a supplement that would restore the birds to a normal weight and hemoglobin level, and if the first supplement, or combination, failed others were added to it. It will be observed that pure vitamins* were frequently supplied along with the crude carriers. At the time these studies were under way, however, many of the vitamins were available only in limited amounts and it was not possible to determine to what extent they could replace the three crude carriers.

After it became evident that a combination of any two of the carriers described is partially incomplete the next step was to combine all three fractions. As shown in Table 3, the combination of the

*Generously supplied by Merck and Co., Rahway, New Jersey.

TABLE 2.—ANY COMBINATION WHICH DID NOT INCLUDE ALL THREE CRUDE VITAMIN CARRIERS WAS INADEQUATE FOR PIGEONS.

Supplement	No. of pigeons	Normal weight gms.	Depletion Period			Experimental Period				
							Weight		Hemoglobin	
			Days	Initial Weight per cent	Initial Hemoglobin per cent	Days	Initial per cent	Final per cent	Initial per cent	Final per cent
TA[1] + F	4	343	163	76	105	38	71	92	63	101
	4	338	103	72	103	20	69	78	61	32
TA + F + inositol	3	365	110	70	100	20	63	71	60	45
LE	3	317	52	74	62	40	70	86	50	62
TA + **LE**	2	368	111	76	97	42	70	96	55	100
LA + F + B_6 + NA	3	328	85	73	85	122	75	88	59	65
LF + F + B_6	4	326	41	72	83	93	77	102	45	102
	2	361	92	76	99	80	68	90	55	89
CA+F+B_6+NA + choline	1	377	40	83	92	40	76	92	52	61
CE+F+B_6	1	287	82	72	78	30	83	98	59	65
TA+**LA**+F	1	321	114	74	106	14	72	81	65	27
TA+**LA**+B^6 +F+NA	1	324	221	79	91	42	71	103	84	110
	1	282	96	87	81	49	82	99	60	77
	1	335	96	66	78	49	74	84	27	55
TA+**LA**+B_6+ F+NA+PA	5	335	95	79	81	99	72	95	54	71
TA+**LF**	1	309	94	62	106	42	60	85	83	105
TA+**LF**+F	3	350	97	75	101	39	70	97	56	95
TA+**CE**+B_6+ F+NA+PA	1	361	185	94	93	63	77	100	55	100
LA+**CA**+F +B_6+NA	2	321	88	79	77	56	74	103	51	91
	2	334	102	74	76	49	70	93	55	91
	2	344	102	74	85	36	65	83	69	96

[1]Symbols for the crude vitamin carriers are in heavy type.

three fractions seemed to be entirely adequate. It will be noted that one pigeon only attained 90 per cent of a normal hemoglobin level, and another only attained 93 per cent of normal weight, but it is not certain that the discrepancies are significant. It frequently happens that when a pigeon is refed on a normal diet after an experimental period, in order that it may recover, its weight and hemoglobin percentage may be either a little higher or a little lower than the original levels. The hemoglobin level in normal pigeons is variable but the average on the Newcomer scale is approximately 100.

At this stage our original objective had been attained. Three components, suitable for the preparation of more concentrated fractions, had been prepared which seemed completely adequate as a source of the unrecognized water-soluble vitamins. The number of these is unknown but, as will be brought out more clearly later, it is quite certain that pyridoxine and pantothenic acid are largely responsible for the effectiveness of these fractions. The fuller's earth adsorbates supply pyridoxine, and the charcoal adsorbate, or eluate, supplies pantothenic acid. There is no intimation of the number of unrecognized vitamins they supply.

Recognized Vitamins Required by Pigeons

Up to this point the vitamins available at the time these studies were underway had been supplied at random, in an attempt to find a combination that is adequate for the pigeon. Damoshek and Myerson ('40) also attempted to determine whether a deficiency of any of the recognized vitamins was responsible for the anemia they observed, but supplied the vitamins one at a time. Completely negative results were obtained with riboflavin, nicotinic acid, vitamin B_6, the "Filtrate Factor" (Lepkovsky) and the "chick-dermatitis factor" (Elvehjem). Yeast extract and certain extracts of liver cured the anemia completely.

Our data have been reorganized to sift out any evidence they afford on the indispensability of specific vitamins. The requirements for single vitamins are shown in Table 4, and selected examples are shown in more detail in Fig. 1. When riboflavin alone was supplied it seems quite certain that the loss in weight and in blood hemoglobin was delayed, but there was no improvement. In certain cases, however, after some of the other supplements had failed riboflavin was superimposed on them. When added to a mixture of Fraction LF and pyridoxine the birds finally attained normal levels both in weight and in blood hemoglobin. However the following paragraph on pyridoxine makes it seem improbable that this combination is consistently effective. It has been our invariable experience that the anemia is cured by tikitiki alone, consequently when tikitiki was

TABLE 3.—THE THREE CRUDE VITAMIN CARRIERS TOGETHER ARE ADEQUATE FOR PIGEONS.

Supplement	No. of pigeons	Normal weight gms.	Depletion Period			Experimental Period				
							Weight		Hemoglobin	
			Days	Initial Weight per cent	Initial Hemoglobin per cent	Days	Initial per cent	Final per cent	Initial per cent	Final per cent
TA+LA+CA +F+NA	2	335	129	80	93	70	72	101	82	101
TA+LA+CA +B_6+F+NA	5	341	105	77	83	80	69	98	66	97
TA+LA+CA +F+NA+PA	1	345	125	80	80	84	82	99	63	90
TA+LA+CA +B_6+F+NA+PA	1	349	136	79	82	98	64	93	42	99
TA+LA+LF +F	1	332	114	71	95	49	69	97	65	115
TA+LA+CE +F+NA+PA	1	360	75	81	94	84	66	102	34	99
TA+LA+CE +B_6+F+NA+PA	1	375	89	77	99	47	71	102	63	100

fortified with riboflavin nothing could be learned of the relation of the vitamin to this type of anemia. Of the six pigeons which received the combination 6 finally attained normal weight, though only after a period of 80 days. A seventh declined slightly in weight during a period of 98 days. Evidently the combination is slightly unsatisfactory. Carter and O'Brien ('37) interpreted their data as demonstrating that the pigeon requires riboflavin.

When pyridoxine alone was supplied, 4 pigeons gave no response and 1 improved both in weight and in hemoglobin level. When superimposed on Fraction LF there was no response in weight, but there was a marked though incomplete response in hemoglobin level. The group which received pyridoxine superimposed on a mixture of Fraction LF and riboflavin is an excellent example of the disturbing variability encountered. Two responded in hemoglobin level but not in weight. Two others responded sharply in weight but only slightly in hemoglobin level. The remaining two recovered completely in hemoglobin level, and almost completely in weight. Carter and O'Brien ('39) have demonstrated that the pigeon requires vitamin B_6.

When pantothenic acid alone was supplied there was no reason to believe it improved the ration. When superimposed on a mixture of other vitamins and crude carriers, 4 pigeons made some improvement, either in weight, in hemoglobin level, or in both. Another continued to decline in hemoglobin level, though at the end its weight was almost normal.

When nicotinic acid alone was supplied to pigeons there was no definite response, though it may be this supplement retarded the decline. Our data on the addition of nicotinic acid to other supplements are not entirely convincing. In no case was this addition made to the diet of pigeons which were markedly low either in weight or in hemoglobin level. In two instances, however, there was some response in hemoglobin level, and in all 4 cases there was some response in weight. There is one additional fragment of evidence which may indicate that nicotinic acid is indispensable. Two pigeons collapsed and lay on the floor of their cage as if completely unconscious. Each was treated with nicotinic acid and each made an almost immediate recovery.

Their histories in brief are given below.

PIGEON 1475

4-28 Weight 318 gms. placed on Ration 1669.
5-26 Weight 222 gms. Given 50 mcg. B_1 daily.
7-8 Weight 217 gms. Collapsed. Mucus membranes of mouth covered with white pustules. Given 5 mg. of nicotinic acid, able to stand within 2 hours.
7-10 Weight 219 gms. Normal except on low plane of nutrition.

TABLE 4.—OBSERVATIONS ON INDIVIDUAL VITAMINS.

Supplement	No. of pigeons	Normal weight gms.	Depletion Period			Experimental Period				
							Weight		Hemoglobin	
			Days	Initial Weight per cent	Initial Hemoglobin per cent	Days	Initial per cent	Final per cent	Initial per cent	Final per cent
(F)[1]	3	338	70	78	91	100	82	72	80	46
LF+B_6+(F)	2	306	49	70	52	65	78	100	72	100
TT+(F)	6	332	44	73	46	80	80	100	100	101
	1	287	54	72	44	98	77	73	100	100
(PA)	2	349	97	80	94	54	80	79	62	44
TA+LA+B_6	1	350	17	64	42	66	62	100	39	79
+F+NA+(PA)	3	331	47	70	68	52	87	96	70	72
	1	328	51	85	30	42	92	95	75	58
(B_6)	4	328	59	69	81	65	73	73	71	53
	1	352	64	67	81	35	70	88	64	90
LF+(B_6)	2	306	25	70	52	25	79	78	53	72
LF+F+(B_6)	2	363	28	80	38	63	78	95	83	112
	2	361	46	68	55	28	73	90	84	89
(NA)	2	277	9	74	84	12	93	80	69	68
LA+(NA)	1	362	50	72	47	28	97	104	89	106
TA+LA+F+(NA)	1	354	56	62	55	56	82	101	85	101
TA+F+(NA)	1	363	77	68	78	32	91	100	106	105
TA+LA+(NA)	1	363	29	76	72	34	80	95	101	104
(NA+B_6)	1	313	45	71	74	15	70	76	55	35
(Choline)	3	367	44	83	84	13	70	68	80	69
(Choline+F+ B_6+NA+CA)	1	377	40	83	92	40	76	92	52	61

[1]The supplements indicated by parenthesis were supplied last, during the experimental period. The supplements not marked in this way were supplied during the depletion period. If all the supplements are in the parenthesis thiamin alone was supplied during the depletion period.

PIGEON 418

5-12 Weight 361 gms. Placed on Ration 1669.
6-11 Weight 250 gms. Given 50 mcg. B_1 daily.
9-4 Weight 210 gms.
2:00 P. M., prostrate on floor of cage, white pustules on mucus membrane of throat. Given 13 mg. nicotinic acid. 10:00 P. M. much improved.
9-5 In fair condition.

Though the data we have indicate that nicotinic acid is required by the pigeon, and the two recoveries mentioned were quite spectacular, we hesitate to accept these observations as conclusive. Only 2 of 275 pigeons, observed over a period of 3 years, have ever developed the symptom of collapse, and there has been no opportunity to determine whether nicotinic acid has any specific activity. Another complication is the possibility that one of the crude vitamin carriers supplied in these trials may contain nicotinic acid. According to Macrae and Edgar ('37) their yeast eluate contains nicotinamide. Presumably then it was present in the adsorbates which we supplied, along with nicotinic acid.

Other investigators in this field have concluded that pigeons do require nicotinic acid. Funk and Funk ('37) state that the pigeons which received nicotinic acid, and especially nicotinamide, consumed more food and attained higher weights than the controls. Harris ('39) stated unequivocally that nicotinic acid is essential for the pigeon. When supplied with a modified Goldberger diet the birds lost weight and collapsed. The addition of 10 mg. daily of nicotinic acid apparently restored normal weight.

Carter and O'Brien ('39) obtained in some cases more complete weight restoration in pigeons by including nicotinic acid with the other supplements, and seemed to regard this vitamin as essential. In another paper, however, presumably written at a later date ('39) they stated that they obtained no response on administering nicotinamide to depleted birds. Nicotinic acid, or the amide, was apparently not responsible for any of the activity of their vitamin B_3 or B_5 concentrates.

Choline alone was supplied to 3 pigeons, and in a complex combination it was supplied to a fourth. In no case was there any response. We do not interpret this as showing that choline is entirely inert when supplied to pigeons, as it is quite possible that there were other first limiting factors.

It will also be observed, as shown in Table 2 especially, that various combinations of pure vitamins, either alone or in addition to crude carriers, have been supplied to pigeons. The pure vitamins in

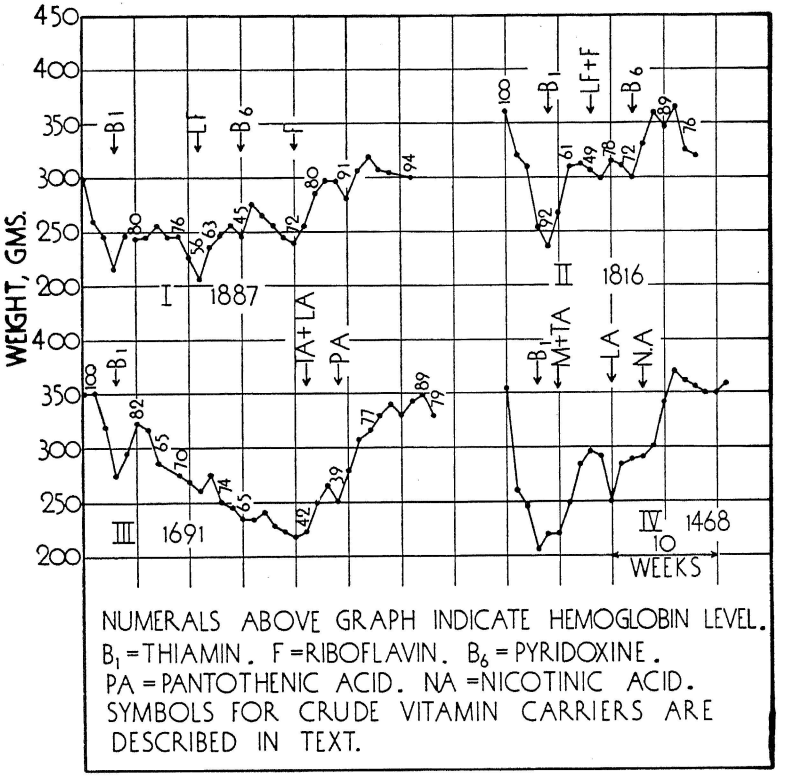


Fig. 1. Pigeon 1887 regained normal weight when supplied with riboflavin. A supplement of pyridoxine raised the level of hemoglobin in the blood of Pigeon 1887, but did not restore the loss in weight. This supplement raised the levels of both hemoglobin and weights in Pigeon 1816, but the improvement was temporary.

A supplement of pantothenic acid definitely raised the level of hemoglobin in the blood of Pigeon 1691, and probably raised the weight level also. It is doubtful that the recovery would have been permanent.

Pigeon 1468 gave some evidence that nicotinic acid is required for the maintenance of normal weight, but the data on that point are not consistent.

these combinations, and the number of pigeons which received them, are shown below.

9, riboflavin + pyridoxine
2, riboflavin + inositol
2, riboflavin + Nicotinic acid
2, pyridoxine + nicotinic acid.
11, riboflavin + pyridoxine + nicotinic acid
2, riboflavin + pantothenic acid + nicotinic acid
1, riboflavin + nicotinic acid + inositol
1, riboflavin + pyridoxine + nicotinic acid + choline
6, riboflavin + pyridoxine + pantothenic acid + nicotinic acid

On the last combination, which is the most complete tried, one of 6 made a complete recovery. There were a few scattered recoveries on the other combinations, but in no case were the recoveries consistent. None of the combinations was as complete as is possible now, but so far as our data go they indicate that pigeons require at least one vitamin which has not yet been recognized as such. As to the recognized vitamins, thiamin requires no comment. It seems equally certain that pigeons require riboflavin, pyridoxine, and pantothenic acid. A deficiency of riboflavin will lead to loss in weight. A deficiency of either pyridoxine or pantothenic acid will lead to anemia and a loss in weight. Our data offer some evidence that pigeons require nicotinic acid, but they are not satisfactory on that point. No positive evidence was obtained that pigeons require choline or inositol but under the conditions they were supplied negative evidence is of doubtful significance.

By way of comment on vitamins B_3 and B_5, it may be well to review briefly their method of preparation, and their activity. Carter and O'Brien ('39) state that vitamin B_5 is an eluate of a fuller's earth adsorbate of yeast or liver extract, and vitamin B_3 is a concentrate prepared from Peter's eluate. We now know that B_5 is an excellent source of pyridoxine, and B_3 of pantothenic acid. The administration of the eluate elicited a marked, but usually incomplete, response in weight. When administered alone the concentrate was only slightly effective. When both were administered normal weight was restored. Crystalline vitamin B_6 was almost as effective as the eluate, and this vitamin is undoubtedly required by pigeons. The authors were of the opinion, however, that some other factor was responsible for the greater effectiveness of the eluate, and considered the possibility that it is nicotinic acid.

According to our experience both vitamin B_3 and B_5 are multiple in nature. The first response to B_3 is explained by its content of pantothenic acid, to B_5 by the presence of pyridoxine. Other vitamins essential for the pigeon are probably present in both B_3 and B_5 but their identity has not yet been determined.

SUMMARY

1. The pigeon-anemia previously described, and loss in weight, were the result of a multiple deficiency.

2. In the absence of either pyridoxine or pantothenic acid pigeons did not recover from anemia.

3. Thiamin, riboflavin, pyridoxine and pantothenic acid are all required for weight maintenance.

4. Evidence that nicotinic acid is required was not entirely conclusive. There was no indication that pigeons require choline or inositol. None of the combinations of pure vitamins tried was entirely adequate.

5. Three crude vitamin carriers were prepared which together contain all unrecognized vitamins required by the pigeon.

6. Vitamins B_3 and B_5 are both multiple. The activity of B_3 is due primarily to pantothenic acid, of B_5 to pyridoxine.

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