

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF EMORY UNIVERSITY.]

**THE PRESENCE OF FOOD ACCESSORIES IN URINE, BILE
AND SALIVA.**

By A. M. MUCKENFUSS.

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The problem of the vitamins has been attacked from several standpoints. Among these is that of metabolism, manifestly difficult of investigation. The next best thing, however, to having direct information on this line is, after studying the foods, to examine the excretions and compare results. Cooper¹ first showed that feces contained the food accessories. He found that 69.1 g. of dried dog's feces, extracted with alcohol and the extract evaporated and concentrated in the usual manner, would cure a pigeon of acute polyneuritis rapidly, but 23 grams of the feces were not quite sufficient. Three and one-half days' feces of a rabbit, treated likewise, also effected a cure. He thought the vitamins of excreta to be derived from undigested food and from bacteria of the colon. It appeared to the writer that this supposition was only partly true. The vitamins are in blood, milk, and in liver and kidney tissue. They appear to be comparatively simple chemical compounds and ought to pass through in part with the secretions.

In 1916 Seidell² worked out what is probably at present the best method of concentrating these substances from solutions, using "autolyzed yeast filtrate" treated with fuller's earth. The writer utilized throughout this series of tests a sample of hydrous aluminum silicate, known as "Lloyd's alkaloidal reagent," kindly furnished by Mr. J. U. Lloyd.³ For brevity it will be referred to as a variety of fuller's earth. Several investigators have noted that it possesses no antineuritic properties. The method of activation was that adopted by Seidell as follows: The mixture of Lloyd's reagent and fresh filtered secretion was well shaken and allowed to stand several hours until clear. The liquid was siphoned off and treated with another portion of the earth, if it was suspected that all vitamins had not been adsorbed during the first treatment. The first portion of "activated earth," left after the above siphoning, weighing from one to four grams, was shaken up with 500 or 1000 cc. of 0.01 *N* hydrochloric acid, according to the amount of earth present. The mixture was allowed to settle clear and the acid siphoned off, when the treatment with very dilute acid was repeated. The sediment of activated earth was now thrown on a Büchner funnel, washed with water, and finally with 3 small portions of 95% alcohol. The alcohol was pressed out with filter paper and the cake dried for an hour on blotting paper. Finally it was dried in a vacuum desiccator, powdered, and bottled for use.

¹ E. A. Cooper, *J. Hyg.*, **14**, 12 (1914).

² A. Seidell, *U. S. Pub. Health Repts.*, **31**, 364 (1916).

³ J. U. Lloyd, *J. Am. Pharm. Assoc.*, **5**, 381, 490 (1916).

Several methods have been proposed to prove the presence of vitamins and to show the comparative amounts present. Eijkman's original plan was to cure fowl (or pigeons) of acute polyneuritis by treatment *per os*. This plan was followed by the original investigators and is still in use. It was employed by the writer. The method is somewhat crude, as McCollum and Simmonds,¹ Funk² and Williams³ have pointed out, but it is well adapted to a preliminary research. The ideal method of investigating nutrition does not seem to have yet been found. No histological examinations were made in connection with this work. The degenerated condition of the nerves, which brings on an acute attack of polyneuritis gallinarum, is now well established, though McCollum and Simmonds⁴ infer that permanent recovery should be effected in order to be sure that the nervous tissue has been regenerated. Weight records were not kept; for as McCollum and Kennedy⁴ suggested, varying loss in weight of pigeons does not seem to affect their recovery from acute attacks of polyneuritis.

Experimental.

The following 3 preparations were used:

1. Activated earth from bile. 5 to 10 liters of fresh bile were obtained under proper supervision from ox bladders and filtered at once, as explained below. The filtrate was agitated with the fuller's earth, 0.4 g. for each 1000 cc. of bile.

2. Activated earth from urine. From 2 to 17 liters of fresh urine, furnished by students, were at once carefully made neutral to litmus with sodium hydroxide and filtered as stated below. The filtrate was shaken with 0.2 to 0.25 g. of fuller's earth for each 1000 cc. of urine.

3. Activated earth from saliva. Saliva from 30 students during 3 hours' collection amounted to about 1300 cc. This was filtered during an interval of about 6 hours in a battery of ordinary filters, and the filtrate shaken thoroughly with the earth, 0.5 to 6.0 g. on different occasions for each 1000 cc. of saliva.

The activated earth was purified by the method of Seidell, outlined above. All pigeons were identified by foot bands, numbered at random as given in the tables below. In general, each was kept on a diet of polished rice until it showed the acute symptoms of polyneuritis, *i. e.*, inability to hold up the head, or to stand, the bird mostly sitting back or falling over. These symptoms can be seen approaching, as is well known. If in the case of a sick bird they were delayed much more than a day, it was treated as soon as convenient without waiting for the acute

¹ Simmonds, *J. Biol. Chem.*, **33**, 55 (1918).

² C. Funk, *Biochem. Bull.*, **4**, 306 (1915).

³ R. R. Williams, *J. Biol. Chem.*, **29**, 495 (1917).

⁴ E. V. McCollum and C. Kennedy, *Ibid.*, **24**, 491 (1916).

TABLE I.—TREATMENT WITH ACTIVATED EARTH FROM BILE.

Laboratory No. of pigeon.	Polyneuritic symptoms when treated.	Days previously on rice diet.	Dose of earth in grams.	Bile equivalent in cc.	Remarks at end of rice diet.	Record after treatment.			Results at that time.
						Days to end of rice diet.	Days before second acute attack.	Days before symptoms grew worse.	
17c	Acute	23	0.7	1750	Flying around for 5 days	6	6	5	Much improved
16c2	Acute	39	0.7	1750		6	..	6	Much improved
33a	Acute	10	0.5	1250		2	Much improved
30a	Acute	28	0.5	1250	Slightly worse	2	..	1	Much improved
13d	Acute	52	0.7	1750	Died. Rain reached room the day before				
17b	Acute	35	0.38	950		2	..	1	Much improved
7f	Acute	21	0.5	1250		1	Much improved
8f2	Acute	34	0.5	1250		1	Improved
26a	Partial, with complications	48	0.38	950	Died	1	..	0	No improvement
12b	Acute, with complications	57	0.7	1750	Died	1	..	0	Worse

TABLE II.—TREATMENT WITH ACTIVATED EARTH FROM URINE.

Laboratory No. of pigeon.	Polyneuritic symptoms when treated.	Days previously on rice diet.	Dose of earth in grams.	Urine equivalent in cc.	Remarks at end of rice diet.	Record after treatment.			Results at that time.
						Days to end of rice diet.	Days before second acute attack.	Days before symptoms grew worse.	
14c	Acute	41	1.2	6000		12	12	5	Much improved
10c	Acute	23	1.2	6000	Flying a little for 7 days	8	..	8	Much improved
6e	Acute	33	0.83	4150		6	6	6	Much improved
16b	Acute	36	1.1	5500		2	Much improved
3f	Partial, with complications	25	0.51	2050		1	..	0	Worse

TABLE III.—TREATMENT WITH SEPARATE SAMPLES OF ACTIVATED EARTH FROM SECRETIONS THAT HAD BEEN EXTRACTED ONCE.

Laboratory No. of pigeon.	Polyneuritic symptoms when treated.	Days previously on rice diet.	Dose of earth in grams.	Record after treatment.			
				Equivalent in cc.	Remarks at end of rice diet.	Days to end of rice diet.	Results.
16c	Acute, with complications	48	0.7	3750 bile	Flying occasionally	5	Much improved
20b	Partial, with complications	52	0.59	2950 bile		1	Much improved
12c	Acute	40	0.65	3250 bile	Died	1	Steadily worse
14c	Acute	36	1.5	15,000 urine	Died	4	Steadily worse

TABLE IV.—TREATMENT WITH ACTIVATED EARTH FROM SALIVA.

Laboratory No. of pigeon.	Polyneuritic symptoms when treated.	Days previously on rice diet.	Dose of earth in grams.	Saliva equivalent in cc.	Record after treatment.			Results at that time.
					Remarks at end of rice diet.	Days to end of rice diet.	Days before symptoms grew worse.	
14b	Acute	39	2.3	466	Died	4	3	Much improved
8f	Acute	33	0.66	1325	Flying a little	1	..	Much improved
4f	Acute	33	2.0	400		1	..	Improved

TABLE V.—RECORD OF CONTROL PIGEONS—NOT TREATED.

Laboratory No. of pigeon.	Polyneuritic symptoms when observed.	Days previously on rice diet.	Record after treatment.			
			Remarks at end of rice diet.	Days to end of rice diet.	Days before symptoms grew worse.	Results at that time.
36a	Acute	41	Died	1	0	Steadily worse
33a2	Partial, with complications	44	Died	1	0	Steadily worse
28a	Acute	24	Much worse for 3 days. Died	4	1	Slightly better
26a2	Acute	31	Worse after one day's improvement. Died		1	Much improved

phase; for in the case of a certain proportion of the birds, the symptoms of polyneuritis do not become aggravated and the animal dies, apparently from refusal to eat. An occasional acute case appeared at the end of such a period. Birds showing "partial symptoms, with complications" or "acute symptoms, with complications" (see tables) do not seem to be fair material for curative tests. Some seem to have kept themselves alive by obtaining bits of feces.

In the tables above, "improved" means ability to manage the head and walk; "much improved," to fly with ease also, when necessary. After the period of observation had passed each bird was given yeast and strengthened on a diet of mixed grain until disposed of. In the tables, some pigeons, which were observed only for a day or two, represented the earlier tests. No pigeon was put on the rice diet a second time, and only full-grown animals were used. On account of the limited amount of activated earth available, it was thought that more definite results would be obtainable by treating several pigeons rather than by attempting to cure the same bird repeatedly, since it might not respond equally well to repeated doses. Most of the pigeons were kept in a clean cage, 32 by 52 by 86 in. When one got to the quiet stage, it was removed to a smaller cage or given the run of a clean concrete room, 15 by 18 by 14 ft., well ventilated. The other pigeons were kept in cages, 13 by 20 by 46 in., two birds in a cage. The cages had double floors and were kept clean. The diet was ordinary polished rice, though this seemed to vary in its inadequacy as a food. Granite grit was on hand; also fresh water.

Preventive Tests.—Two pigeons on a polished rice diet showed no beneficial symptoms from treatment *per os* with 10 to 15 cc. of fresh filtered saliva twice daily; two gave similarly negative results from eating polished rice thinly coated with "activated earth from saliva." Length of observation, 27 days.

Summary.

1. Nine pigeons with acute symptoms of polyneuritis on a diet of polished rice were treated *per os* with fuller's earth activated with 950 to 1750 cc. of fresh filtered ox bile. Eight of these showed improvement while observed, two for 5 and 6 days, respectively.

2. Four pigeons with acute symptoms of polyneuritis were given fuller's earth activated with 4150 to 6000 cc. of fresh filtered human urine. All improved greatly, 3 of them for 5, 6 and 8 days, respectively, one of these having been observed for 12 days before suffering a second acute attack.

3. Three pigeons with acute symptoms of polyneuritis were treated with different samples of fuller's earth that had been mixed with 2950 to 3250 cc. of the bile filtrate from the above-activated earth. Two improved and one grew worse. One pigeon with acute symptoms was given

fuller's earth treated with 15,000 cc. of the urine filtrate from the above activated earth, but continued to grow worse.

4. Three pigeons with acute symptoms of polyneuritis improved after treatment with fuller's earth, activated with 400 to 1325 cc. of fresh filtered human saliva. Simple preventive tests with saliva were negative.

5. Four "control" pigeons, which acquired the acute symptoms of polyneuritis on a diet of polished rice along with the others above, were not treated. All grew worse, 3 dying within a day.

Conclusions.

1. These experiments indicate that the antineuritic vitamine is probably present in comparatively small quantity in clean, fresh, filtered bile from the bladder of the ox.

2. This food accessory is also present in fresh filtered human urine in traces apparently.

ATLANTA, GEORGIA.

NOTE.

Fischer's Theory of Water Absorption in Edema.¹—Professor Henderson has again called attention, in a recent number of *THIS JOURNAL*,² to the fundamental requirements which Fischer's theory of water absorption by protoplasm³ fails to satisfy, or even to consider. Emphasis is justly laid upon the fact that no significant swelling of the protein blocks used by Fischer has been shown to occur within the range of acidities possible in the body fluids of living mammals.² It might be answered, although the point seems not to have been closely considered, that within the cell acidities may prevail different from those known in blood, cerebrospinal fluid, and similar juices. There is reason to suppose that the reaction of intracellular fluids is, as a rule, less alkaline than that of blood, but the basis for this belief has not been extensive. Without venturing to add unduly to the already voluminous discussion of the "colloid-chemical" theory of water metabolism, attention may, nevertheless, be called to some evidence regarding the range of intracellular acidities obtaining in the living tissues of animals.

This evidence is derived from cases in which pigments capable of behaving as sensitive indicators of acidity have been found within the cells of certain marine invertebrates.⁴ The hydrogen-ion concentrations at which these indicators change color were determined by appropriate

¹ Contributions from the Bermuda Biological Station for Research, No. 94.

² Henderson and Cohn, *THIS JOURNAL*, 40, 857 (1918); Henderson, Palmer and Newburgh, *J. Pharmacol.*, 5, 449 (1914).

³ "Edema and Nephritis," 2nd Ed., New York, 1915; Fischer and Hooker, *THIS JOURNAL*, 40, 272 (1918).

⁴ Crozier, *J. Biol. Chem.*, 24, 255, 443 (1916); 35, 455 (1918).