LXXIII. THE PRESENCE OF THE ANTINEURITIC AND ANTISCORBUTIC VITAMINS IN URINE.

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(*Received July 29th, 1922.*)

FUNK [1914], SEIDELL [1921] and others applied chemical methods in order to determine the structure of the antineuritic vitamin. Their efforts to isolate these mysterious substances were not perfectly successful and therefore we do not wonder that nothing is known as yet of the metabolism of these factors. However, recently some experiments have been made to examine the excretion of antineuritic vitamins from the organism.

Cooper [1914] succeeded in curing polyneuritis-pigeons with an alcoholic extract of faeces.

Muckenfuss [1918] tried to find antineuritic vitamins in ox-bile, human urine and saliva by shaking up these substances with fuller's earth. The fuller's earth, treated in this way, purified with water and alcohol and then dried, proved to be efficient in curing pigeons with polyneuritis.

Gaglio [1919] at Rome also investigated the result of administering urine to polyneuritis-pigeons. For this purpose he concentrated the urine and found that even 3–4 cc. were able to cure the birds. Urine of rabbits that had fasted for 15–20 days was likewise active, though less than that of normal rabbits which were fed on cabbage leaves and bran. The ash of urine proved to be without action.

Gaglio [1919] as well as Curatolo [1920] are convinced that these cures are not to be attributed to the action of vitamins but to the influence of non-specific products of metabolism. On this supposition is founded the research of di Matteï [1920], who tried to cure pigeons with coffee. Indeed, 5 % of mocha proved to have a strong curative power, but to his astonishment he perceived that pure caffeine (in non-toxic doses) had only a slight effect.

Funk [1912] had previously demonstrated that some purine and pyrimidine derivatives had a more or less favourable influence on polyneuritis.

It has also been proved that a mixture of NaCl and KCl may have a good, though temporary effect on the disease [Eijkman, van Hoogenhuyze and Derks, 1922].

Still more remarkable is the observation made by Theiler who described the favourable result of subcutaneous injection of distilled water [1921].

It is difficult to explain this effect from a stimulation of vitamins, still present in the organism. Theiler attributed this action to "*spontaneous recovery.*"

Jansen [1920], in the Medical Laboratory at Weltevreden (Java), confirmed the observations of Eijkman and Theiler.

Yet, the results described by Gaglio and Curatolo are so striking, that we doubt whether such a lasting and absolute recovery may be attributed to the influence of non-specific products of metabolism.

Funk informs us that the recovery obtained by treatment with hydantoin lasted only nine days, whereas a pigeon treated with thymus-nucleic acid lived on for a fortnight. Allantoin, adenine and other pyrimidine derivatives were less active. From the administration of uric acid he did not obtain any effect.

It occurs to us that much is to be said in favour of the opinion of Muckenfuss who believes antineuritic vitamins to be present in urine.

By a series of experiments on pigeons we tested the opinions of these investigators and tried to find the most probable explanation.

First, 12 pigeons (Nos. 1–12) were fed on polished rice and water till they showed symptoms of polyneuritis. Then 5 cc. of urine that had been concentrated *in vacuo* at 45° were administered *per os.* In six cases this had undoubtedly a favourable influence.

In most cases the symptoms had much improved one day after the administration; sometimes even the influence was evident after a few hours. The body weight, having diminished before the appearance of the clinical symptoms, went up again in all cases of improvement. Yet we did not succeed in regaining the initial weight.

Eight pigeons (Nos. 13–20) were treated prophylactically with the administration of concentrated urine from the very first. The result was that the average incubation period increased from 22 to 42 days.

To enhance, if possible, the curative power of the urine the latter was dried in large open Petri dishes in the incubator at 37°. After about 16 hours a thin layer of tough, semi-solid substance was found which was dried in an exsiccator at room temperature; 1 g. of dried substance was found to be equivalent to 25 cc. of urine with a S.G. of 1·030.

Seven polyneuritis-pigeons (Nos. 21–27) were treated with this dried urine, dissolved in autoclaved milk and administered in quantities of 1·5–2 g. per day. Four of these pigeons were cured rapidly and absolutely. After stopping the administration of dried urine, some days passed before the birds showed further symptoms of the disease. We succeeded in curing a second attack in two pigeons.

Three pigeons (Nos. 28–30) were treated by subcutaneous injection of dried urine, dissolved in sterilised milk. These however died after a few days.

It is well known that strong heating destroys the antineuritic vitamin. We therefore tried the action of urine which had been heated for one hour

at 130°. After sterilisation the urine was filtered, neutralised with hydrochloric acid and then concentrated *in vacuo* to a specific gravity of 1·050.

Ten polyneuritis-pigeons (Nos. 31–40) were treated with 15 cc. of this liquid daily. In one case the disease lasted only seven days, whereas the medicament had not the least effect on the other birds. This proves that by strong heating the curative power of the urine is decreased.

We also investigated the action of urine ash. For this purpose dried urine was incinerated, by which process it lost about 75 % of its weight. Ten pigeons (Nos. 45–54) were treated *per os* with this ash, suspended in water, and it was found to be almost inactive.

Chamberlain and Vedder [1911] observed that the antineuritic factor was removed by filtering through animal charcoal. We made use of this discovery and shook up urine with purified animal charcoal, 200 cc. of urine being shaken up for 45 minutes with 5 g. of animal charcoal; afterwards the urine was centrifuged and the charcoal shaken up anew with 200 cc. of fresh urine. This process was repeated three times. The charcoal was then washed once with distilled water and dried at 37°.

Six polyneuritis-pigeons (Nos. 60–65) were treated with this "*carbo cum urina*," 2 g. of this substance suspended in a little water being administered per day. All the birds recovered within a short time and we kept them healthy for at least a fortnight. The second attack too was cured. The smallest active dose proved to be 1 g.

Crude (non-purified) charcoal, shaken up with urine and treated in the same way, was far less active (Table I).

Table I. *Treatment of polyneuritis-pigeons with carbo cum urina.*

Laboratory no. of pigeon	Incubation period (days)	Period of illness* (days)	Effect of treatment	Dose of *Carbo cum urina*, g.	Remarks	Weight (g.) Beginning of experiment	Weight (g.) End of experiment
					Crude charcoal		
55	16	1	0	2	Died	240	200
56	21	2	0	2	,,	265	175
57	45	1	0	2	,,	240	175
58	22	5	±	2	Tempor. improved	350	225
59	16	12	±	2	,, ,,	260	200
					Carbo anim. puriss.		
60	24	18	+	2	Much improved	295	175
61	17	18	+	2	,, ,,	265	175
62	35	—	+	2	,, ,,	280	220
63	22	30	+	2	,, ,,	380	315
64	14	30	+	2	,, ,,	270	?
65	32	—	+	2	,, ,,	265	?
66	14	1	+	1	,, ,,	?	?
67	18	15	±	1	Slightly improved	355	260
68	17	19	±	0·5	,, ,,	330	290

* The period of illness is counted from the moment the treatment begins until death or, in case of recovery, until the end of experiment.

Another experiment on 12 pigeons (Nos. 69–80) showed that the non-treated animal charcoal had absolutely no effect.

Normal urine, shaken up with a surplus of charcoal, then filtered and concentrated *in vacuo*, had no effect (pigeons Nos. 81–89).

Then eight polyneuritis-pigeons (Nos. 90–98) were treated with animal charcoal, which had been shaken up with urine, heated previously for three hours at 120°. We expected this charcoal to be ineffective. On the contrary, two pigeons recovered and walked normally for ten days: six other pigeons were not cured. Without a doubt the curative effect of this charcoal is far inferior to that of charcoal shaken up with fresh urine. However, it must be admitted that there are some substances present in urine, possessing a curative power, and capable of being adsorbed by charcoal, and yet not bearing the character of the so-called vitamins. We tried to find out whether these substances have an organic or an inorganic character. For this purpose a mixture of inorganic salts was made, approximately in the same proportion as those found in human urine:

$NaCl$	9·2 %	Na_2HPO_4	5·0 %
K_2SO_4	2·7	$MgCl_2$	1·5
$(NH_4)_2SO_4$	1·5	$CaH_4(PO_4)_2$	0·25
KH_2PO_4	2·8		

This solution was shaken up with purified animal charcoal and ten pigeons (Nos. 99–108) were treated with this charcoal, which proved to have a very small effect as was expected beforehand, Rona and Michaëlis [1919] having demonstrated that animal charcoal will only slightly adsorb inorganic salts, the cations and anions present in urine being least adsorbed.

We may therefore safely assume that sterilised urine has a definite curative power owing to the presence of non-specific organic substances.

Finally we made an investigation as to whether the bacilli present in urine had any influence. The urine used was fresh, but not sterile. We did not expect to have a positive result here as the quantity of bacilli as far as weight was concerned, was very small.

Besides it has been already shown that *B. coli* does not contain any antineuritic vitamin even when cultivated in an extract of rice bran [Eijkman, van Hoogenhuyze and Derks, 1922], whilst Damon [1921] demonstrated that the B-vitamin is absent from *B. paratyphosus B.*, *B. coli* and *B. subtilis*. Fresh urine was kept at 37° for 24 hours. The bacilli out of this urine were cultivated on agar, then suspended in distilled water and afterwards heated at 100° for 30 minutes. This suspension of dead bacilli was then filtered through animal charcoal. After this process the latter was dried at 37°. As expected this charcoal proved to have scarcely any curative power (Table II).

Finally the fact that yeast is only active in a medium that contains antineuritic vitamin or its components was made use of [Eijkman, van Hoogenhuyze and Derks, 1922]. Baker's yeast was cultivated at 28° for 24 hours in fresh urine, the acid reaction of which had been diminished and to which had been added 5 % of glucose. This yeast was centrifuged, washed once with distilled water and dried at 37°. It proved to have a strong curative

action on polyneuritis of pigeons, 2 g. of yeast being sufficient to ensure the birds an absolute and lasting cure. This experiment makes it probable that antineuritic vitamins or their components are present in fresh, normal urine.

Table II. *Treatment with bacilli-charcoal.*

Laboratory no. of pigeon	Incubation period (days)	Period of illness (days)	Effect of treatment	Dose of bacilli-charcoal, g.	Remarks	Weight (g.) Beginning of experiment	Weight (g.) End of experiment
109	27	5	±	2	Slightly improved	330	230
110	31	6	±	2	„ „	360	350
111	19	4	0	2	Died	300	190
112	20	5	0	1	„	300	250
113	28	4	0	0·5	„	340	205
114	28	10	±	0·25	Slightly improved	350	200
115	15	7	±	0·25	„ „	325	260
116	19	4	0	0·25	Died	?	?
117	19	4	0	0·25	„	?	?
118	19	4	0	0·25	„	305	240

In order to trace whether diet has any influence on the activity of urine, the urine of a dog also was examined. Charcoal shaken up with this urine proved to possess as curative an action as human urine. Two g. per day were quite sufficient, but one pigeon even recovered from the use of 0·25 g. per day. As long as we were experimenting with its urine, the dog was fed on potatoes, polished rice and meat. This food contains a sufficient quantity of anti-neuritic vitamin. Then the diet was altered by autoclaving all the dog's food (bread, rice, meat and potatoes) during three hours. Only three weeks after the dog had been taking the vitaminless diet, the urine was again examined every day and shaken up with charcoal. The urine proved to have lost all its curative power (Table III).

Table III. *Treatment with charcoal shaken up with urine of a dog.*

A. The dog was fed on normal diet.

Laboratory no. of pigeon	Incubation period (days)	Period of illness (days)	Effect of treatment	Dose of charcoal, g.	Remarks	Weight (g.) Beginning of experiment	Weight (g.) End of experiment
123	13	—	+	2	Much improved	280	230
124	23	—	+	1·5	„ „	340	285
125	28	—	+	1	„ „	380	300
126	25	—	+	1	„ „	380	250
127	42	1	0	1	Died	300	195
128	33	8	±	0·5	Slightly improved	310	290
129	31	1	0	0·5	Died	320	190
130	37	—	+	0·25	Improved	290	245
131	35	8	0	0·25	Died	?	?

B. The dog was fed on a vitamin-free diet.

132	23	6	0	2	Died	320	260
133	28	2	0	2	„	300	220
134	22	4	0	2	„	315	200
135	21	2	0	2	„	360	?
136	26	5	0	2	„	330	?
137	22	6	±	2	Slightly improved	350	250
138	17	2	0	2	Died	300	240
139	26	5	0	2	„	340	205

It became more and more obvious that the favourable result must at least partly be attributed to the presence of the antineuritic vitamin, yet we went on to treat some fowls with urine because up till now it has never been demonstrated that non-specific substances have any influence on the polyneuritis of fowls. We found that fresh, concentrated urine (S.G. 1·050), as well as dried urine, when administered in large quantities is able to cure polyneuritis-fowls. Still more efficient was the action of animal charcoal, shaken up with fresh human urine. This however has to be administered in doses of 15 g. (equivalent to 2·5 litres of urine!) a day. Even then it required ten days to cure the fowl whilst we did not always succeed in curing the symptoms of paralysis absolutely. By a check experiment on six fowls it was shown that the charcoal itself did not exercise any curative action, and a fowl treated with charcoal that had been shaken up with urine, heated previously at 130° for one hour, did not recover.

The result of the experiments on fowls strengthened our opinion as to the presence of a small quantity of antineuritic vitamin in urine.

Antiscorbutic vitamin.

We then examined whether the antiscorbutic vitamin was also present in the urine.

For this purpose some guinea-pigs were put on a scorbutic diet receiving in addition fresh urine *per os*. The standard diet selected was a mixture of oats and bran, which together with water was given *ad lib*. Great care was taken to give the animals the best conditions possible. Following the example of Delf [Delf and Tozer, 1918] five guinea-pigs (Nos. 1–5) (Fig. 1) were given about 60 cc. of milk, previously autoclaved for an hour at 120°. Two other animals (Nos. 6 and 7), kept on the same diet, received daily 10 cc. of urine (which had been diluted to a specific gravity of 1·020). All these guinea-pigs developed scurvy and succumbed after some weeks. By post-mortem histological examination of the costochondral junctions we found the abnormalities described by Tozer [Delf and Tozer, 1918] in the severe stage of scurvy: irregularity of the junction, disorder of the rows of cartilaginous growing cells, usually a well-developed reticular zone, haemorrhages etc.

The average loss of weight of the guinea-pigs which were getting urine came to 29 % as against 19 % of the former group, the average lifetime being 28 days against 27 of the first group.

Evidently the administration of urine had no influence on the severity of the microscopical changes and on the average lifetime, whereas the loss of weight had increased.

(Though we made a histological examination of the ribs of all the guinea-pigs we are conscious that we have to be cautious in judging the results. It is known that Tozer [1921] demonstrated that the lack of A-vitamin may bring about departures from the normal which closely resemble those which are present in chronic cases of scurvy.)

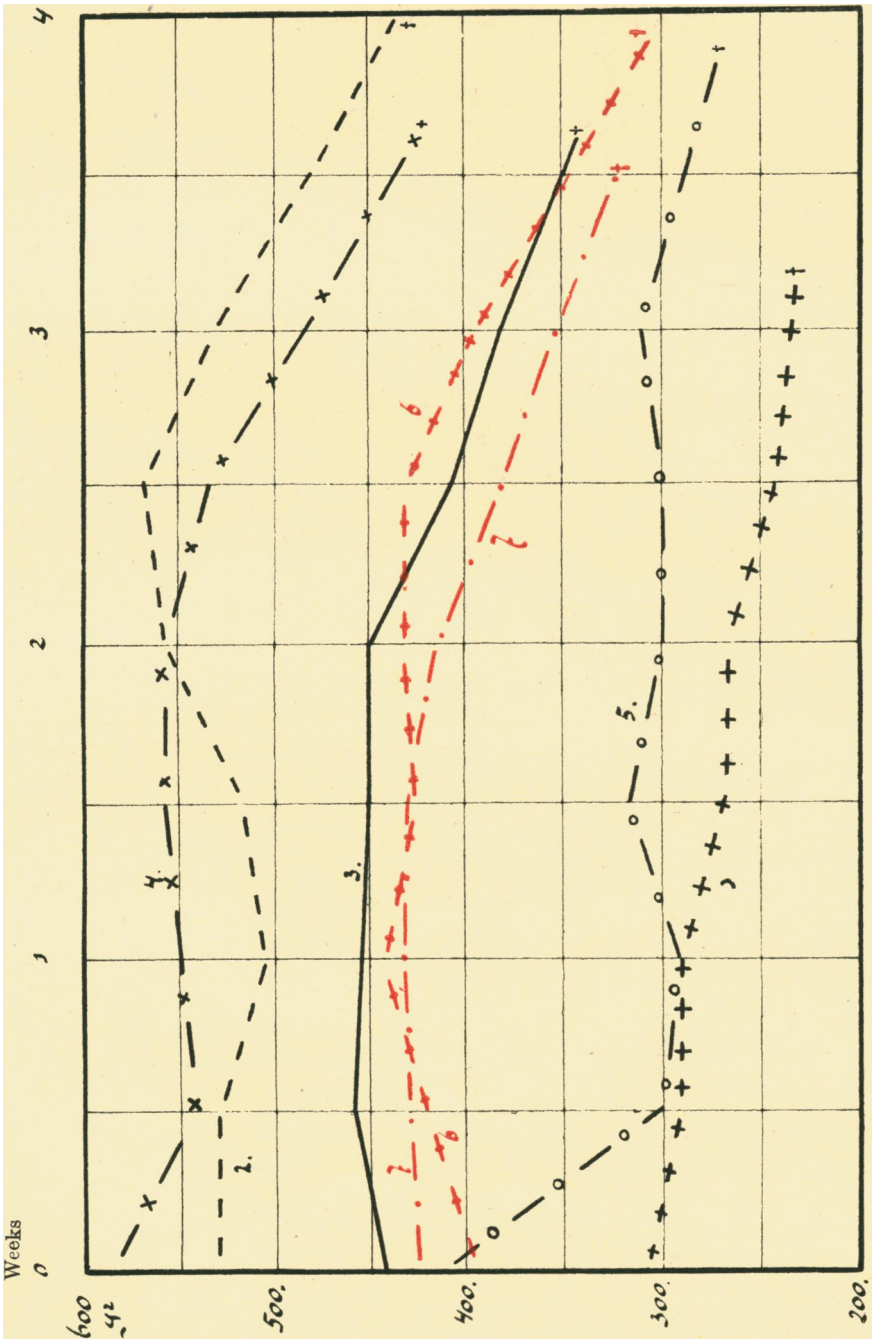

Fig. 1 Experiments I and I *a*.
Guinea-pigs, Nos. 1–5. *Diet:* oats, bran and water; autocl. milk (1 h. at 120°) *ad lib.*
Guinea-pigs, Nos. 6, 7. " " " " +10 cc. of urine.

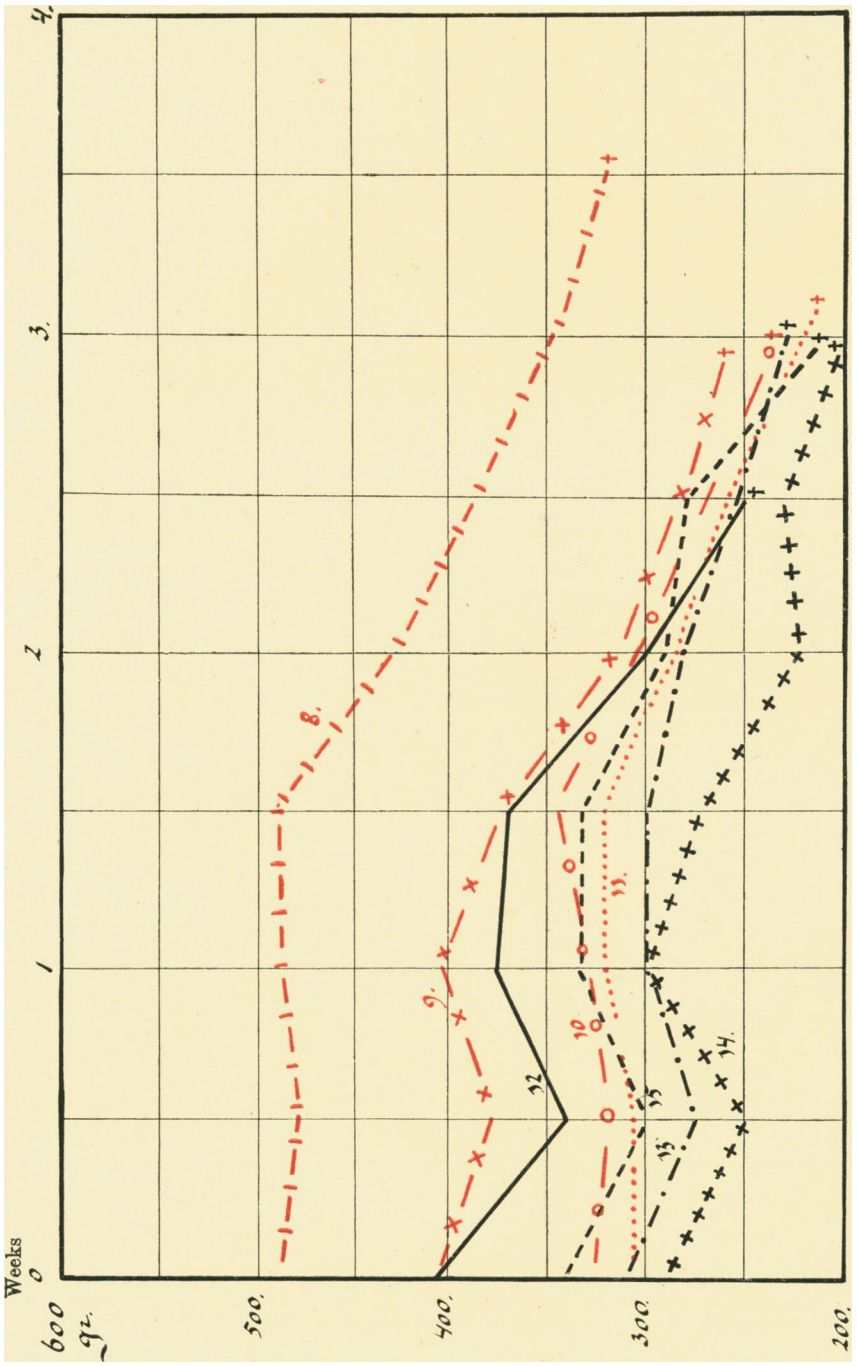

Fig. 2. Experiments II and II *a*.
Guinea-pigs, Nos. 12–15. *Diet*: oats, bran, water, 10 cc. of autocl. milk.
Guinea-pigs, Nos. 8–11. „ „ „ „ + 10 cc. of urine.

It appeared to us that the guinea-pigs were loth to take the autoclaved milk and it was necessary to give an additional quantity with a syringe, which took a great deal of time.

Experiment II. Four guinea-pigs (Nos. 12–15) were given with a syringe 10 cc. of autoclaved milk besides the usual scorbutic diet. Just as had been demonstrated by Delf, it was found that the loss of weight had increased (about 34 %). The average lifetime was about the same (25 days), and the microscopical changes were very similar in both experiments (Fig. 2).

Experiment II a. Another series of animals (Nos. 8–11), fed on the same standard diet, were also given 10 cc. of fresh urine daily. The average loss of body weight was 31 % and the average lifetime was 26 days. The microscopical abnormalities were not less than those in the above experiments.

Experiment II b. In this experiment the amount of urine was increased; 20 cc. being administered instead of 10. The average loss of weight of this group of animals (Nos. 16–19) was 26 %, and the average lifetime was 23 days (Fig. 3). On histological examination we found marked disorganisation of the normal structure. In this experiment too the urine proved to be without antiscorbutic power. (Although the administration of a large quantity of autoclaved milk had a favourable influence on the weight and the general state of the guinea-pigs, we thought it advisable to administer only 10 cc. of autoclaved milk to the animals in the following experiments, by which method it was still possible to make a comparison with the former groups.)

As we did not think it advisable to administer a greater dose of urine, we tried to find out in the following experiments whether urine was able to increase the antiscorbutic power of a small quantity of green cabbage.

Experiment III. Four guinea-pigs were given besides the diet of oats and bran 1 g. of green cabbage. These animals (Nos. 20–23) died in about 33 days, the average loss of weight being 32 %. The macroscopical as well as the microscopical changes were far less than in the former experiments (Fig. 4).

Experiment III a. 1 g. of green cabbage was evidently not quite enough absolutely to prevent the onset of scurvy. We then gave four guinea-pigs (Nos. 24–27) in addition 15 cc. of fresh urine. It was found that the average lifetime of these last animals, as compared to that of those in Expt. III, had increased only two days, the average loss of weight being 24 %. The result of the microscopical examination was similar to that of Group III. (We think the decrease of the average loss of body-weight was only owing to guinea-pig No. 23, the effect of the green cabbage being extraordinarily favourable in this case.) Without a doubt this experiment shows that the antiscorbutic power of 1 g. of green cabbage was great (a well-known fact), but that a dose of 15 cc. of urine was unable to exercise any curative effect.

Experiment IV. A further group of animals (Nos. 28–32) was given, besides the standard diet, 2 g. of green cabbage. By this treatment the average loss of weight was only 17 % whereas the average lifetime increased to 69 days. The influence of this quantity of cabbage proved to be much greater than the

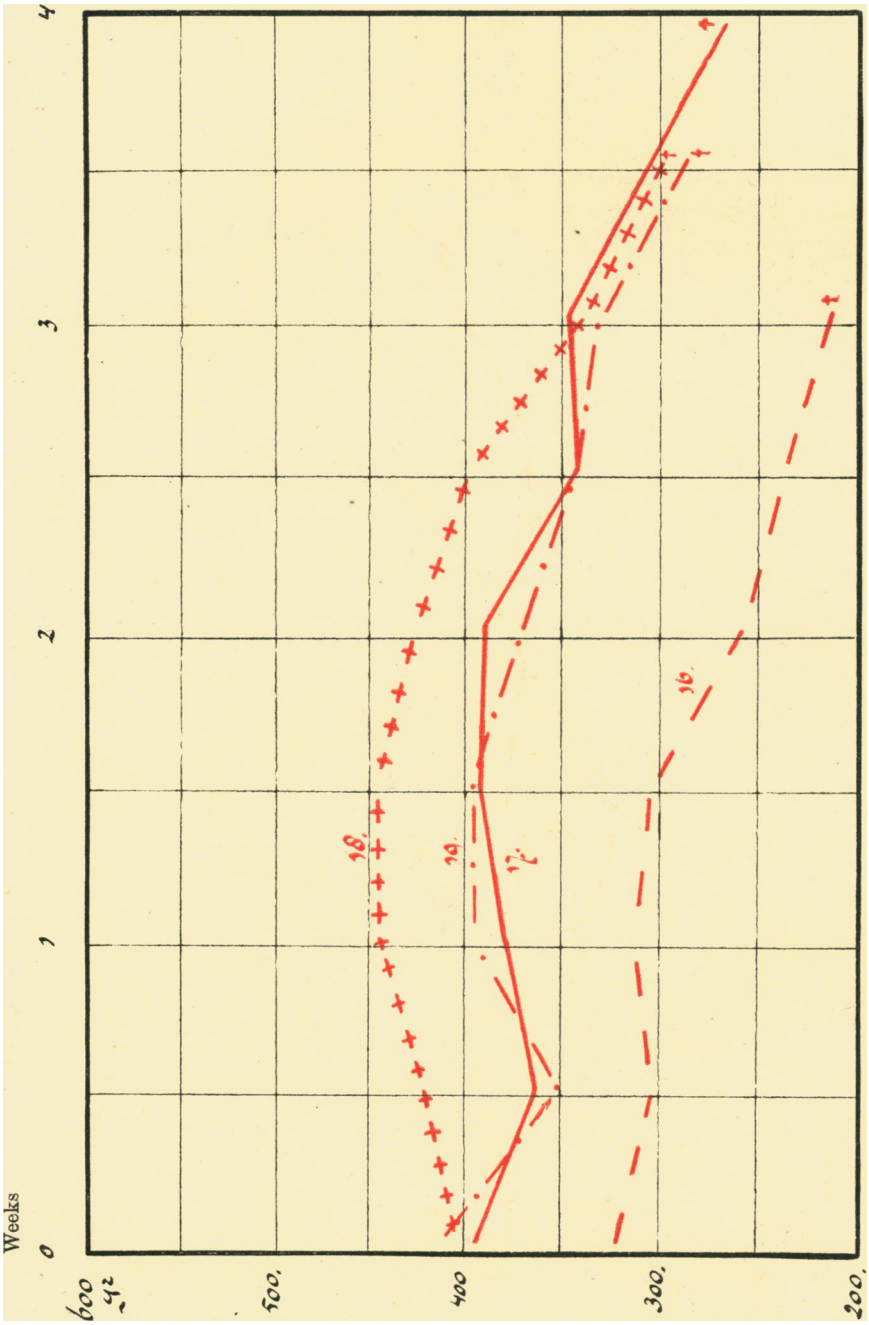

Fig. 3. Experiment II *b*.
Guinea-pigs, Nos. 16–19. *Diet*: oats, bran, water + 10 cc. of autocl. milk + 20 cc. of urine.

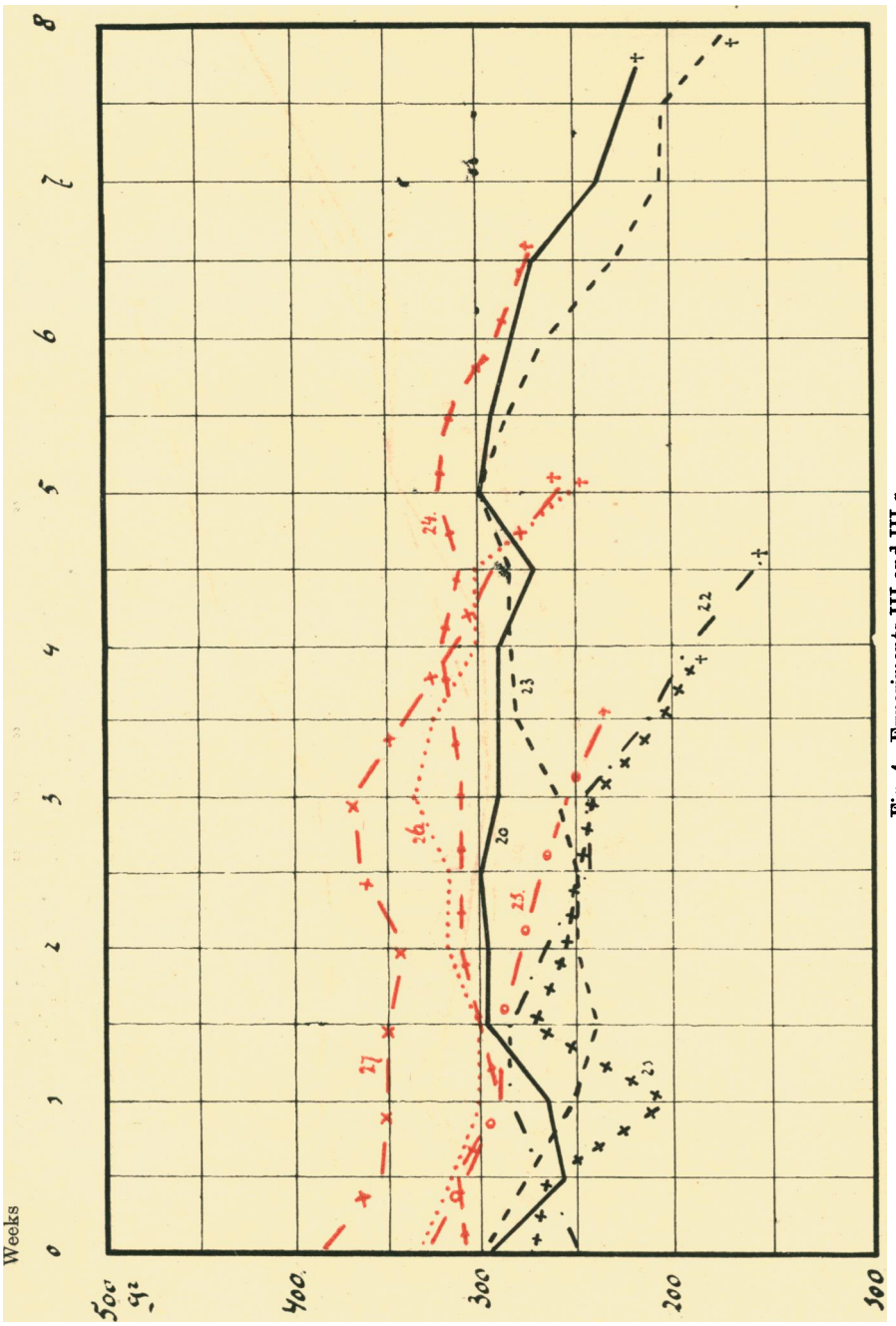

Fig. 4. Experiments III and III *a*.

Guinea-pigs, Nos. 20–22. *Diet*: oats, bran, water + 1 g. of green cabbage.
Guinea-pig, No. 23. " " " + " " + 10 cc. of autool. milk.
Guinea-pigs, Nos. 24–27. " " " + " " + " " + 15 cc. of urine.

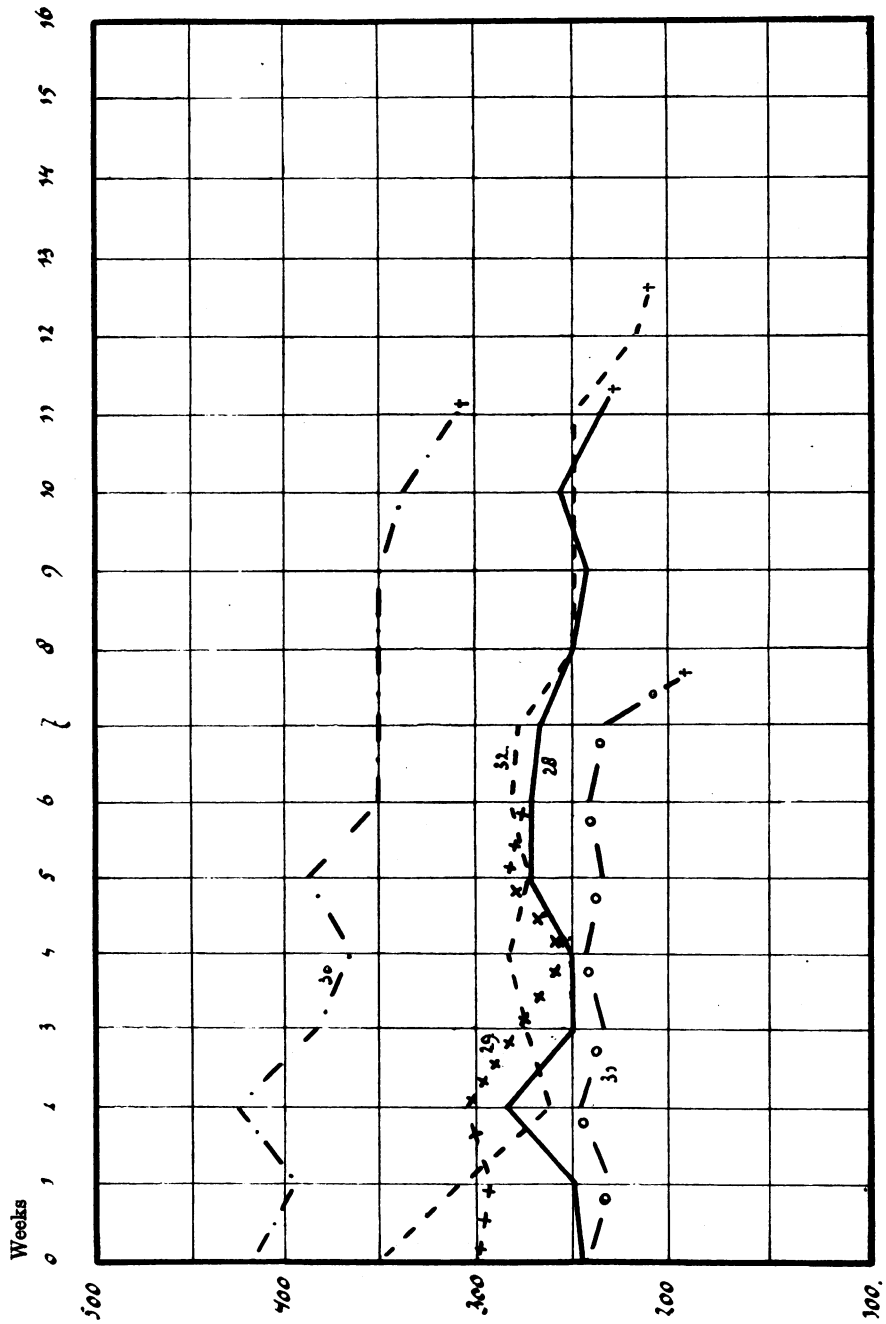

Fig. 5. Experiment IV.
Guinea-pigs, Nos. 28–31. *Diet:* oats, bran, water + 2 g. of green cabbage.
Guinea-pig, No. 32. „ „ „ + „ „ + 10 cc. of autool. milk.

effect of giving a combination of 1 g. of cabbage plus 15 cc. of urine (Fig. 5). The microscopical changes found post-mortem were approximately those described by Tozer in the incipient stage.

In none of these experiments were we able to detect any antiscorbutic power of the urine. Of course it is possible that the urine might prove to be effective if given in greater quantities. This was practically impossible. Theoretically one might try to concentrate the urine just as Harden and Robison [1920] concentrated orange-juice and Bassett-Smith lemon-juice. We did not think it advisable to do this with urine because of the unknown influence of the other substances in urine on the labile antiscorbutic vitamins. Harden and Zilva [1918] demonstrated that charcoal does not adsorb the antiscorbutic vitamin and so we could not use this method of procedure, which proved so successful in the case of the antineuritic vitamin.

Conclusions.

1. It was shown that concentrated, normal urine may exercise a favourable influence on polyneuritis of pigeons.

2. Urine, dried at 37° for 16 hours, was likewise active.

3. By heating the urine at 130° for one hour, the curative power was destroyed.

4. The ash of urine was inactive.

5. *Carbo anim. puriss.*, shaken up with urine, centrifuged, washed with distilled water and then dried, had a strong curative power.

6. Urine, treated with a surplus of charcoal and then filtered, had lost its curative power.

7. Charcoal, shaken up with urine, which had been heated previously for three hours at 120°, was far less active.

8. Charcoal not shaken up with urine proved to be quite inactive.

9. Charcoal, shaken up with a solution of inorganic salts, containing the cations and anions present in urine, had only a slight effect.

10. The curative power exercised by urine may not be attributed to the presence of bacilli.

11. Yeast, cultivated in urine, to which had been added 5 % of glucose, exercised a strong curative action.

12. The urine of a dog had as strongly curative an action as that of man, but when the dog was fed on a vitamin-free diet, the urine lost its curative power.

13. It was shown that in some cases fresh, normal urine or dried urine, when given in large quantities, may have a good influence on polyneuritis of fowls.

14. Charcoal shaken up with normal urine was likewise active.

15. The charcoal itself had no curative influence on the polyneuritis of fowls.

16. Charcoal treated with urine heated previously for one hour at 130° had no effect.

17. It was pointed out that a small quantity of antineuritic vitamin is present in the urine, as proved by the above experiments.

18. We were unable to detect the presence of the antiscorbutic vitamin in urine.

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