ON THE PROTECTIVE AND CURATIVE PROPERTIES OF CERTAIN FOODSTUFFS AGAINST POLYNEURITIS INDUCED IN BIRDS BY A DIET OF POLISHED RICE.

Part II.

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In a previous communication (Cooper, 1913) the amounts of various raw foodstuffs, *e.g.* beef, heart-muscle, brain, fish, egg-yolk, lentils, and barley were set forth which were sufficient to prevent polyneuritis for a definite period in pigeons fed on polished rice.

It was found that, while (ox) cardiac muscle, egg-yolk, lentils, and barley were very efficient in preventing polyneuritis, the voluntary muscle of both ox and fish possessed only feeble anti-neuritic powers. Sheep-brain was less efficient than ox-heart, but more so than beef in preventing polyneuritis.

The relatively small efficiency of brain material suggests that either the anti-neuritic substances, although essential for the integrity of the nervous system, are not present therein in the active condition or they are combined in such a form that they are not readily absorbed from the alimentary canal.

It was also found that, although small amounts of brain material did not prevent polyneuritis, they were effective in checking the loss in weight that usually accompanies the disease. This fact supported the conclusion reached by Schaumann (1911) that the loss in weight was not merely due to the deficiency in polished rice of the anti-neuritic substances, but resulted largely from a secondary deficiency of other substances essential for the maintenance of body-weight.

To obtain more information as to the relative efficacy of various foodstuffs for preventing beri-beri and to ascertain further facts as to

the distribution of the anti-neuritic substances in animal tissues, I have made some more experiments with brain, liver, milk, cheese, nuts, and malt-extract and the results obtained are set forth in the present communication.

These experiments consisted in determining the daily amounts of the above foodstuffs which prevented polyneuritis in pigeons for a definite period. As pigeons develop symptoms of polyneuritis in about three weeks when fed exclusively on polished rice, the standard time selected was 50 days.

Pigeons received daily by artificial feeding constant rations of polished rice equal to 1/20th their initial body-weight and rations different for each bird of the tissue under investigation. All the tissues were thoroughly minced before being fed to the pigeons.

In this way it was possible to determine a maximum daily amount insufficient to prevent polyneuritis for 50 days and a minimum amount sufficient for this purpose. The suitability of the various diets for the maintenance of body-weight was also studied, the birds being weighed weekly for this purpose.

I. *Control experiments with polished rice.*

Seven pigeons were fed artificially on polished rice, the daily ration being 1/20th their initial body-weight. The results of the experiments are set forth below.

TABLE I.

	Pigeon 326	Pigeon 327	Pigeon 328	Pigeon 329
Effect of diet on pigeons	Symptoms of polyneuritis appeared on 23rd day	Symptoms of polyneuritis appeared on 25th day	Symptoms of polyneuritis appeared on 20th day	Symptoms of polyneuritis appeared on 23rd day
Change in weight by end of exp.	− 26 %	− 24 %	− 17 %	− 22 %

	Pigeon 330	Pigeon 133	Pigeon 134
Effect of diet on pigeons	Symptoms of polyneuritis appeared on 22nd day	Symptoms of polyneuritis appeared on 22nd day	Symptoms of polyneuritis appeared on 17th day
Change in weight by end of exp.	− 16 %	− 22 %	+ 3 %

The results indicate that the birds developed symptoms of polyneuritis in about three weeks and at the end of that time had usually lost considerably in weight.

II. *Ox-brain.*

TABLE II.

Series I. (*a*) Cerebellum (water-content 80 %).

	Pigeon 10	Pigeon 11	Pigeon 12	Pigeon 13
Daily ration of natural tissue	4 gms.	5 gms.	7 gms.	9 gms.
Effect of diet on pigeon	Symptoms of polyneuritis on 21st day	Still healthy on 31st day	Still healthy on 31st day	Still healthy on 31st day
Change in weight by end of exp.	0	− 9 %	0	− 12 %

(*b*) Cerebrum (water-content 80 %).

	Pigeon 6	Pigeon 7	Pigeon 8	Pigeon 9
Daily ration of natural tissue	4 gms.	5 gms.	7 gms.	9 gms.
Effect of diet on pigeon	Symptoms of polyneuritis on 22nd day	Still healthy on 31st day	Still healthy on 31st day	Still healthy on 31st day
Change in weight by end of exp.	− 20 %	− 19 %	− 9 %	− 4 %

Series II. (*a*) Cerebellum.

	Pigeon 340	Pigeon 341	Pigeon 342	Pigeon 343	Pigeon 344
Daily ration of natural tissue	3 gms.	4 gms.	6 gms.	9 gms.	12 gms.
Effect of diet on pigeon	Symptoms of polyneuritis on 24th day	Symptoms of polyneuritis on 34th day	Weakness in limbs on 46th day	Died on 39th day with symptoms of weakness	Still healthy after 50 days
Change in weight by end of exp.	− 15 %	− 17 %	− 6 %	− 8 %	− 5 %

(*b*) Cerebrum.

	Pigeon 345	Pigeon 346	Pigeon 347	Pigeon 348	Pigeon 349
Daily ration of natural tissue	3 gms.	4 gms.	6 gms.	9 gms.	12 gms.
Effect of diet on pigeons	Symptoms of polyneuritis on 30th day	Weakness in limbs on 46th day	Still healthy on 50th day	Still healthy on 50th day	Still healthy on 50th day
Change in weight by end of exp.	− 25 %	− 20 %	− 18 %	+ 5 %	− 17 %

The results indicate that the amounts of cerebrum and cerebellum required to prevent the *acute* symptoms of polyneuritis in pigeons fed on polished rice were about equal, but, while 6 gms. of cerebrum were sufficient to keep the birds healthy and strong for 50 days, 12 gms. of cerebellum were necessary to prevent signs of weakness in the later days of the experiment. Cerebrum therefore appeared to contain

a somewhat greater available amount of the anti-neuritic substances than cerebellum. Notwithstanding this however there was a tendency for cerebellum to be somewhat more effective than cerebrum in reducing the loss in weight resulting from the polished rice diet. These observations support the conclusion reached by Schaumann (1911) that the loss in weight accompanying polyneuritis is not entirely an effect of the malnutrition necessarily resulting from the deficiency in the diet of the anti-neuritic substances, but must be largely due to a secondary deficiency in polished rice of substances essential for the maintenance of body-weight. This view was also supported by the fact recorded in the previous communication (1913) that the addition of a small amount of sheep-brain to the polished rice diet, although ineffective in preventing polyneuritis, was sufficient to reduce the loss in weight to a considerable degree. On comparing the results obtained with ox- and sheep-brain however it is found that the former is less efficacious than the latter in this respect, although somewhat more potent in preventing polyneuritis. No explanation of this can at present be advanced.

III. *Ox-liver.*

(*Water-content* 70 %.)

TABLE III.

	Pigeon A	Pigeon 1	Pigeon 2	Pigeon B	Pigeon 3
Daily ration of natural tissue	1 gm.	2 gms.	3 gms.	4 gms.	5 gms.
Effect of diet on pigeons	Symptoms of polyneuritis on 8th day	Symptoms of polyneuritis on 47th day	Still healthy on 50th day	Still healthy on 35th day	Still healthy on 31st day
Change in weight by end of exp.	− 9 %	− 13 %	− 8 %	− 6 %	− 11 %
	Pigeon 4	Pigeon 5	Pigeon 83	Pigeon 84	Pigeon 85
Daily ration of natural tissue	7 gms.	12 gms.	3 gms.	4 gms.	5 gms.
Effect of diet on pigeons	Still healthy on 31st day	Still healthy on 31st day	Still healthy on 50th day	Still healthy on 50th day	Still healthy on 50th day
Change in weight by end of exp.	− 3 %	− 3 %	− 28 %	− 13 %	0
	Pigeon 86	Pigeon 87	Pigeon 88		
Daily ration of natural tissue	7 gms.	9 gms.	12 gms.		
Effect of diet on pigeons	Still healthy on 50th day	Still healthy on 50th day	Still healthy on 50th day		
Change in weight by end of exp.	− 12 %	− 12½ %	0		

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The results indicate that 3 gms. of ox-liver daily were sufficient to prevent polyneuritis in pigeons for 50 days, and 2 gms. delayed the development of the disease until the 47th day of the experiment. The richness of liver in the anti-neuritic substances suggested that the tissue might even be effective in curing polyneuritis. Accordingly, minced ox-liver was dried at 30° C. by an electric fan, ground, and the dry powder emulsified with water and administered to neuritic pigeons. It was found that 7 gms. of dried liver (21 gms. liver in natural condition) rapidly cured the pigeons and prevented the reappearance of the disease for one week, while 4 gms. ameliorated the symptoms but could not effect complete recovery. Cod-liver oil in doses ranging from 2 to 8 gms. however possessed no curative properties.

IV. *Cow's milk.*

(*Water-content* 87 %.)

Vedder and Clark (1912) found that of four fowls fed on polished rice and 5 c.cs. of fresh cows' milk daily two developed symptoms of polyneuritis in a short time, while the remaining two were healthy even after two months.

In the following experiments the fresh milk was well shaken in a machine immediately before use and in the case of the larger rations of milk the birds were fed four times daily.

TABLE IV.

	Pigeon 69	Pigeon 70	Pigeon 71	Pigeon 72
Daily ration of milk	2 c.cs.	3 c.cs.	4 c.cs.	6 c.cs.
Effect of diet upon birds	Severe weakness in limbs on 38th day	Symptoms of polyneuritis on 18th day	Symptoms of polyneuritis on 11th day	Severe weakness in limbs on 38th day
Change in weight by end of exp.	− 24 %	− 24 %	− 6 %	− 26 %
	Pigeon 74	Pigeon 57	Pigeon 52	Pigeon 53
Daily ration of milk	12 c.cs.	15 c.cs.	20 c.cs.	20 c.cs.
Effect of diet upon birds	Symptoms of polyneuritis on 18th day	Weakness in limbs on 16th day	Symptoms of polyneuritis on 33rd day	Symptoms of polyneuritis on 36th day
Change in weight by end of exp.	− 12 %	− 6 %	− 12 %	− 26 %
	Pigeon 54	Pigeon 55	Pigeon 138	Pigeon 153
Daily ration of milk	35 c.cs.	35 c.cs.	10 c.cs.	30 c.cs.
Effect of diet upon birds	Symptoms of polyneuritis on 49th day	Symptoms of polyneuritis on 54th day	Symptoms of polyneuritis on 11th day	Symptoms of polyneuritis on 19th day
Change in weight by end of exp.	− 20 %	− 12 %	− 18 %	− 16 %

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It is thus seen that cow's milk possessed only feeble anti-neuritic properties, as much as 35 c.cs. daily merely delaying the development of polyneuritis until about the 50th day of the experiment. The birds receiving diets of polished rice and milk also suffered considerable loss in weight.

V. *Nuts* (*Husked filberts*).

(*Water content* 4 %.)

TABLE V.

	A		B	
	Pigeon 264	Pigeon 265	Pigeon 267	Pigeon 268
Daily ration of nuts	1 gm.	1 gm.	2 gms.	2 gms.
Effect of diet on pigeon	Symptoms of polyneuritis on 20th day	Symptoms of polyneuritis on 36th day	Still healthy on 50th day	Still healthy on 50th day
Change in weight by end of exp.	− 25 %	− 10 %	− 14 %	− 8 %

	C		D	
	Pigeon 269	Pigeon 270	Pigeon 271	Pigeon 272
Daily ration of nuts	3 gms.	3 gms.	5 gms.	5 gms.
Effect of diet on pigeon	Still healthy on 50th day	Still healthy on 50th day	Still healthy on 50th day	Still healthy on 50th day
Change in weight by end of exp.	0	− 11 %	+ 7 %	+ 13 %

	E	
	Pigeon 273	Pigeon 274
Daily ration of nuts	7 gms.	7 gms.
Effect of diet on pigeon	Still healthy on 27th day	Still healthy on 27th day
Change in weight by end of exp.	+ 10 %	+ 8 %

The results indicate that the daily addition of 2 gms. of husked filberts to the polished rice diet was sufficient to prevent polyneuritis in pigeons. For the maintenance of body-weight 5 gms. of nuts daily were necessary. As it was previously found (Cooper, 1913) that 3 gms. of lentils or 4 of husked barley were required to prevent polyneuritis, it appears that nuts are somewhat superior to these foodstuffs in anti-neuritic power.

VI. *Cheese.*

Finely-ground cheddar cheese (water-content 30 %) was employed in the experiments.

TABLE VI.

	A		B	
	Pigeon 275	Pigeon 276	Pigeon 277	Pigeon 278
Daily ration of cheese	1 gm.	1 gm.	2 gms.	2 gms.
Effect of diet on pigeon	Symptoms of polyneuritis on 14th day	Symptoms of polyneuritis on 20th day	Symptoms of polyneuritis on 31st day	Died on 38th day with symptoms of weakness.
Change in weight by end of exp.	− 8 %	− 17 %	− 10 %	− 19 %
	C		D	
	Pigeon 279	Pigeon 280	Pigeon 281	Pigeon 282
Daily ration of cheese	4 gms.	4 gms.	8 gms.	8 gms.
Effect of diet on pigeon	Symptoms of polyneuritis on 22nd day	Died on 38th day with symptoms of weakness	Symptoms of polyneuritis on 15th day	Died on 28th day with symptoms of weakness.
Change in weight by end of exp.	− 18 %	− 10 %	0	0

The results indicate that the addition of as much as 8 gms. of cheese daily to the polished rice diet failed to prevent polyneuritis. Thus, while 35 c.cs. of fresh milk daily (Table IV) delayed the appearance of polyneuritis until the 50th day of the experiment, the equivalent amount of cheese (3·5 gms.)[1] and even more than twice this amount had no preventive effect. Possibly the anti-neuritic substances are destroyed during the process of cheese-ripening or they may be less readily absorbed from cheese than from milk.

VII. *Malt Extract.*

Experiments were carried out to ascertain whether malt extract possessed curative properties against polyneuritis. Three samples of the extract were employed. Two of them were found to exert a rapid curative action upon neuritic pigeons, but a third sample even in large doses had no effect. The results of the experiments are briefly set forth below.

Sample I (Water-content 24 %)
Minimum curing dose = 5 gms.

„ II (Water-content 27 %)
Minimum curing dose = 7 gms.

„ III (Water-content 18 %)
10 gms. had no curative action.

[1] 100 parts of milk yield approximately 10 parts of cheese.

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Discussion of Results.

In the following table the minimum amounts of the various foodstuffs required to prevent polyneuritis in pigeons fed on polished rice are compared.

Foodstuffs	Amounts necessary to prevent polyneuritis: In terms of natural foodstuff	In terms of dry-weight
Ox voluntary muscle (1)	20 gms.	5·0 gms.
Ox cardiac muscle (1)	5 ,,	1·7 ,,
Ox cerebrum	6 ,,	1·2 ,,
Ox cerebellum	12 ,,	2·4 ,,
Ox liver	3 ,,	0·9 ,,
Cow's milk	>35 ,,	>3·5 ,,
Sheep cerebrum (1)	8 to 15 ,,	1·6 to 3 ,,
Fish voluntary muscle (1)	>10 ,,	>2 ,,
Egg-yolk (1)	3 ,,	1·5 ,,
Lentils (dry) (1)	—	3 ,,
Barley (husked) (1)	—	4 ,,
Nuts (husked filberts)	—	2 ,,
Cheese	>8 ,,	>5·6 ,,

(1) Cooper, 1913.

The results indicate that the various ox-tissues are not of equal anti-neuritic power, liver being most effective in preventing polyneuritis, then cardiac muscle and cerebrum, next cerebellum, and least effective, voluntary muscle and cow's milk. This order still obtains when the results are expressed in terms of dry material.

Liver is thus considerably more efficient in preventing polyneuritis than either cerebrum or cerebellum. In the natural condition cardiac muscle is about as efficient as cerebrum, but somewhat more so than cerebellum ; on comparing the dried materials however heart-muscle is of smaller anti-neuritic efficacy than cerebrum, but still retains its superiority to cerebellum.

Before attempting to draw conclusions from the above results as to the actual distribution of the anti-neuritic substances amongst animal tissues and fluids, however, it is necessary to determine to what extent the various food-materials are absorbed from the alimentary canal of birds. In man egg-yolk and voluntary muscle (both of ox and fish) can be almost completely absorbed, but liver and cardiac muscle, owing to their denser structure, are less readily digested (Hutchinson, 1900). As however in my experiments these tissues were well minced, there is no reason to suppose that absorption would be less complete. Brain material, on the other hand, is imperfectly absorbed, as much as 40 %

appearing in the faeces, and this may explain its relatively small efficacy in preventing polyneuritis in birds.

I have not determined the proportion of these various foods absorbed by pigeons. It would involve a very large number of experiments to obtain results of any value, but experiments to determine the proportion absorbed when brain in various amounts is fed to birds are in progress.

The whole content of anti-neuritic substances is not absorbed even from a normal diet of grain, as it was possible to detect the presence of these substances in the excreta of a hen fed on maize, barley, and buckwheat. The active substances could also be detected in the faeces of a rabbit fed on white bread and cabbage. The excreta of the bird were collected daily for a fortnight and dried at 30° C. by an electric fan. The total amount of dry material obtained was 150 gms. This was extracted repeatedly at 35° C. with absolute alcohol in a shaking machine, the filtered extract concentrated *in vacuo* and freed from alcohol. The residue was a black gummy substance weighing 6½ gms. Three gms. of this, equivalent to one week's excretion, administered orally to a pigeon affected with polyneuritis rapidly exerted a complete curative action and the bird again fed on polished rice remained well for a week. One gram of the extract, equivalent to two days' excretion, improved the condition of other neuritic birds, but did not effect complete recovery. The rabbit's faeces were collected daily for a week and dried at 30° C. as above. The total amount of dry material was 75 gms. This was extracted with alcohol, the procedure being similar to that employed in the case of the bird's excreta, and one half of the total amount of dry alcoholic extract, equivalent to about three days' excretion, rapidly cured a pigeon affected with polyneuritis, but within twenty-four hours the bird again became ill.

The anti-neuritic substances present in the excreta may be derived not merely from the diet, but to some extent possibly from the bacteria growing in the large intestine. Yeast is known to be particularly rich in the active substances, so that it may be reasonably supposed that these substances are also synthesised by bacteria.

Experiments carried out to ascertain whether *B. coli* contains any considerable amount of anti-neuritic substance have been made but the extract from 2 gms. of bacteria was without effect. It is evident that the amount contained is much less than in yeast.

The results, so far, afford indications as to the composition of diets suitable for the prevention of beri-beri.

Of animal tissues heart-muscle, liver, and egg-yolk are much more valuable for this purpose than voluntary muscle of either fish or ox and, if reckoned as dry weight, are somewhat superior even to lentils, nuts, and barley, which are suitable vegetable foodstuffs to supplement the polished rice diet. The small value of flesh as a prophylactic against polyneuritis which emerges from my experiments on birds is borne out by practical experience.

According to Van Leent (1880) prior to 1876 the native crews of the Dutch East Indian Navy received a diet of polished rice (75 %) and meat or fish (25 %) and suffered considerably from beri-beri, while the European crews whose dietary contained in addition beans, peas, potatoes, and greens were almost free from the disease. Subsequently the native crews drew the same rations as the Europeans and as a result of this change there was a great fall in the number of beri-beri cases.

In 1902 and 1903[1] the native troops in the Philippines owing to the prevalence of cholera were prevented from obtaining a supply of vegetables from the markets and were consequently restricted to a diet of polished rice and meat. Soon after this change beri-beri broke out and a large proportion of the Company were affected, although for a whole year before the troops had been in excellent health.

The substitution of heart-muscle and liver for ordinary flesh in the mixed diets employed in localities where beri-beri occurs would thus be a distinct improvement, as, not only are the former tissues when properly prepared as nutritious as voluntary muscle, but they also contain higher available concentrations of the anti-neuritic substances.

Summary.

1. Pigeons fed on daily rations of polished rice equal to 1/20th their initial body-weight develop symptoms of polyneuritis in about three weeks and usually lose considerably in weight.

2. The efficacies of various ox-tissues for preventing polyneuritis have been determined, and the tissues arranged according to their anti-neuritic powers are in the following descending order: liver, cardiac-muscle, cerebrum, cerebellum, voluntary muscle, and (cows') milk.

3. Alcoholic extracts of the excreta of a hen fed on unpolished grain and of the faeces of a rabbit fed on bread and cabbage cured polyneuritis in pigeons. The whole content of anti-neuritic substances

[1] *Report Surg.-Gen. Army, U.S.A.* 1902–1903, p. 69.

present in the dietary was therefore not absorbed or else some amount is synthesised by the bacteria of which the faeces consisted to a considerable extent. No conclusions can consequently be drawn as to the actual distribution of the active substances in the animal body, until the extent to which the various tissues are absorbed from the alimentary canal of birds has been determined.

4. Nuts (husked filberts) are very efficient in preventing polyneuritis, being even superior to lentils and husked barley. Cheddar cheese, on the other hand, even in considerable amount, has no preventive effect.

5. Malt extract taken from two different samples readily cured polyneuritis in pigeons. A third sample however even in large doses had no curative action.

6. For the prevention of beri-beri egg-yolk, heart-muscle, liver, nuts, barley, and lentils can be recommended as suitable foodstuffs with which to supplement the polished rice diet. As meat (voluntary muscle) has been frequently found to be ineffective in preventing epidemics of beri-beri, its replacement by heart and liver in mixed diets would be a considerable improvement, because, not only are these tissues when suitably prepared as nutritious as voluntary muscle, but they also contain the anti-neuritic substances in much higher concentration.

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