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

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The historical aspect of beri-beri has recently been so exhaustively dealt with by Schaumann (1910), Simpson and Edie (1911), and Funk (1912), that it is only necessary to consider here in detail the previous investigations of the protective and curative effects of various foodstuffs and their extracts towards this disease and the analogous polyneuritis of birds.

The evidence obtained by Eijkman (1897) and Braddon conclusively proved that beri-beri occurred amongst those races that used a highly polished (steam-milled) rice as their staple diet, but not amongst those peoples whose diet consisted of rice decorticated by hand or parboiled rice.

Fletcher (1907) by the application of this discovery found that beri-beri disappeared from amongst the inmates of an asylum subsequently to the replacement of polished rice by whole rice as the staple diet.

Eijkman (1897) first shewed that a disease (polyneuritis) similar in many respects to human beri-beri could be induced in birds by polished rice diets. He furthermore found that fowls developed symptoms of this disease when fed almost exclusively on starch (tapioca, sago, etc.) and that the birds recovered after receiving raw meat.

Eijkman (1906) also found that an aqueous extract of rice-polishings cured polyneuritis after the removal of the phytin and that the curative constituent was dialysable and was not precipitated from aqueous solution by alcohol. More recently (1910) he has shewn that the curative substance of yeast is also extracted by strong alcohol.

Gryns (1900, 1909) confirmed many of Eijkman's observations and also shewed that the addition of katjang-idjoe beans and meat to the

polished rice diet prevented polyneuritis. He found, however, that their protective action was destroyed by heating to 120° C.

Schaumann (1910) shewed that the addition of proteins, glycerophosphates and inorganic salts to the polished rice diets did not prevent polyneuritis in pigeons and that the addition of sodium nucleate only delayed the occurrence of the symptoms and decreased their severity. He compared quantitatively the preventive powers of dried brewers' yeast, rice-bran and wheat-bran against pigeon polyneuritis and found that the daily addition of 1·5, 2 and 7·5 gms. respectively of these substances to the polished rice diet protected the birds from this disease and led to an increase in their weights.

Schaumann also shewed that impure yeast-nucleic acid and egg-lecithin possessed slight curative properties towards polyneuritis, but could not bring about the complete recovery of the birds, while dried yeast, peas, and katjang-idjoe beans were able to restore completely birds affected with this disease. The birds could also be cured by hydrochloric acid (0·3 %) and alcoholic (96 %) extracts of these beans.

Fraser and Stanton (1910, 1911) extracted the constituent of rice-bran, which cured polyneuritis in pigeons, by dilute hydrochloric acid and absolute alcohol, and Chamberlain and Vedder (1911) further shewed that the curative substance could be removed from the dried alcoholic extract of rice-bran by water and that it was dialysable, thus confirming Eijkman's observations of years before.

Chamberlain and Vedder also found that fowls, receiving daily in addition to the polished rice diet sufficient of this aqueous extract, did not develop polyneuritis and that the extract contained only traces of phosphorus ($\frac{1}{1000}$ th part of the phosphorus-content of rice-polishings). This result lent no support to the theory that beri-beri is due to a deficiency of phosphorus in the rice diet which had been advanced in more than one quarter.

Cooper and Funk (1911) confirmed many of the previous observations and found that polyneuritis could be induced in birds by diets of various pure carbohydrates, thus excluding intoxication as a cause of beri-beri. They also found that the anti-neuritic constituent of rice-polishings after extraction with acidulated alcohol and water was completely precipitated by phospho-tungstic acid and was a nitrogenous body which contained no phosphorus.

Funk (1911) has continued these fractionations and has found that after precipitation with phospho-tungstic acid and decomposition of the

precipitate with baryta the curative constituent of polishings is completely precipitated by silver nitrate, partially by mercuric chloride in alcoholic solution and not at all by platinum chloride in alcoholic solution and is a crystalline body melting at 233° C. Funk has provisionally ascribed the formula $C_{17}H_{19}O_7N_2$ to the active substance.

Although Chamberlain and Vedder (1911) found that lime-juice itself did not cure polyneuritis, Funk in a later communication (1912) shewed that by concentration and fractionations similar to those described previously by him (1911) a fraction could be obtained from the lime-juice with silver nitrate possessing marked curative properties. Curative fractions were also obtained from ox-brain, yeast and milk.

Schaumann (1912) has confirmed some of the main observations of Funk and suggests that the anti-neuritic substance is an activator by which the assimilation of phosphorus is rendered possible, so that under dietetic conditions leading to the depletion in the organism of the active substance phosphorus starvation takes place.

B. Moore and his collaborators (1912) have also confirmed some of Funk's observations and have employed similar methods for the isolation from yeast of the anti-neuritic substance to which they ascribe provisionally the formula $C_7H_{17}N_2O_5$.

The investigation about to be described may be divided into two parts.

Part I deals with the quantitative determination of the preventive powers of various common foodstuffs against polyneuritis induced in birds by diets of polished rice.

Part II deals with the fractionation of some of these foodstuffs carried out with a view to the isolation of the active constituent or constituents.

Part I of the investigation was undertaken not merely on account of its intrinsic interest to dietetics, but also because of its practical importance in dealing with beri-beri. The active substance has, I believe, already been isolated by Funk at this Institute, but it exists in such minute amount and is so difficult to separate that its extraction in the pure condition for addition to a diet otherwise inadequate is not yet a practical proposition. It is likely that when its precise constitution has been determined, it may be discovered how to synthesise it but this will probably occupy some considerable time.

In the meanwhile knowledge of the relative available concentration of the active substance in certain common foodstuffs which can be used to supplement the diets of coolies will surely be of value.

Part I.

The distribution of the substance or substances preventing polyneuritis amongst foodstuffs.

The amounts of various foodstuffs have been determined which are necessary to prevent the occurrence of polyneuritis for a definite time in adult pigeons of an average weight of 350 gms. fed on polished rice[1]. As pigeons fall ill with polyneuritis in about three weeks when fed exclusively on polished rice, the standard time selected was 50 days.

Series of pigeons received in addition to the rice diet a certain ration of a foodstuff daily. The latter was administered by a crop-tube and differed in amount for each series. In this way it was possible to determine a maximum daily amount of a foodstuff insufficient to prevent polyneuritis in pigeons for 50 days and a minimum amount sufficient for this purpose. The symptoms of polyneuritis, induced by an exclusive diet of polished rice or by mixed diets consisting of polished rice and insufficient rations of the various foodstuffs, were accompanied by fatty degeneration in the sciatic nerves which was demonstrated by means of Marchi's method. No relation was observed between the degree of degeneration and the intensity of the paralytic symptoms.

The birds were weighed weekly in order to compare the influence of the various mixed diets upon their body-weights.

The anti-neuritic values of the following raw foodstuffs have been examined in this way:

1. Meat (lean beef).
2. Ox-heart.
3. Sheep-brain.
4. Fish (hake).
5. Egg-yolk.
6. Brewers' yeast.
7. Lentils.
8. Barley.

Control experiments with polished rice.

Nine pigeons (Series I) were fed exclusively on polished rice the daily ration being $\frac{1}{20}$th their body-weight. For the first two weeks of

[1] Daily ration of rice $= \frac{1}{20}$th body-weight approx. During the first two weeks of an experiment the birds ate the rice naturally, but subsequently they grew tired of the diet and were fed artificially.

the experiments the birds ate the rice readily, but subsequently they became tired of the diet and ate very small amounts of rice, the daily ration being much less than $\frac{1}{20}$th.

A second series of pigeons received by regular artificial feeding *throughout* the experiment daily rations of polished rice of from $\frac{1}{11}$th to $\frac{1}{30}$th the initial body-weight.

The results of these experiments are set forth below :

TABLE I.

Series I.

Natural feeding.

	Pigeon 1	Pigeon 2	Pigeon 3	Pigeon 4	Pigeon 5
Effect of diet upon pigeons' health	Weakness appeared on 18th day. Acute symptoms of polyneuritis on 24th day.	Symptoms of polyneuritis on 21st day.	Symptoms of polyneuritis on 22nd day.	Symptoms of polyneuritis on 32nd day.	Symptoms of polyneuritis on 33rd day.
Mean change in weight by end of a certain time	$-24{\cdot}6$ % after 18 days				

	Pigeon 35	Pigeon 38	Pigeon 39	Pigeon 41
Effect of diet upon pigeons' health	Symptoms of polyneuritis on 21st day.	Symptoms of polyneuritis on 28th day.	Symptoms of polyneuritis on 26th day.	Symptoms of polyneuritis on 33rd day.
Change in wt. by end of exp.	$-18{\cdot}7$ %	-36 %	-30 %	$-10{\cdot}7$ %

Series II.

Artificial feeding.

(*a*)

Daily ration = $\frac{1}{15}$th body-weight.

	Pigeon 181	Pigeon 182	Pigeon 184
Effect of diet upon pigeons' health	Symptoms of polyneuritis on 14th day.	Symptoms of polyneuritis on 13th day.	Symptoms of polyneuritis on 10th day.
Change in weight by end of exp.	$+3$ %	$-7{\cdot}7$ %	-6 %

(*b*)

	Pigeon 241	Pigeon 240	Pigeon 239	Pigeon 236
Daily ration of rice in fractions of Initial body-weight	1/30th	1/23rd	1/19th	1/17th
Daily ration of rice in fractions of Final body-weight	1/24th	1/15th	1/14th	1/13th
Day of exp. upon which symptoms of polyneuritis appeared	16th day	19th day	15th day	16th day
% change in weight by end of exp.	$-21{\cdot}1$ %	$-35{\cdot}2$ %	$-23{\cdot}7$ %	-23 %

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	Pigeon 233	Pigeon 238	Pigeon 243	Pigeon 244
Daily ration of rice in fractions of Initial body-weight	1/14th	1/14th	1/11th	1/11th
Daily ration of rice in fractions of Final body-weight	1/13th	1/12th	1/9th	1/10th
Day of exp. upon which symptoms of polyneuritis appeared	16th day	13th day	26th day	9th day
% change in weight by end of exp.	−7 %	−14·3 %	−22·4 %	−13 %

The results indicate that the pigeons fed naturally and therefore on small rations of polished rice developed symptoms of polyneuritis from three to four weeks after being placed on the rice diet and by that time had suffered a loss in weight which varied from 10 to 36 %.

In the case of the birds receiving the larger rations by regular artificial feeding the symptoms of polyneuritis appeared much earlier. No appreciable difference, however, was noticed in the periods elapsing before the appearance of polyneuritis in the case of pigeons receiving artificially daily rations of rice varying from $\frac{1}{11}$th to $\frac{1}{30}$th the initial body-weight. The explanation of this may be that the birds eating small insufficient rations of polished rice depend upon their own tissues for making up the deficiency in the food supply. In this way they are more able than the well-fed birds to make use of the anti-neuritic substance distributed throughout their bodies and thus remain free from polyneuritis for a longer period.

Similar experiments carried out by other workers have, however, yielded most divergent results: Maurer (1907) found that fowls which ate polished rice in the largest amount were the first to fall ill with polyneuritis, and that this disease was postponed for a long time in under-fed birds. The experiments described above confirm Maurer's results. Chamberlain, Bloombergh and Kilbourne (1911), on the other hand, found that birds, which ate their full daily ration of rice, remained healthy for a longer period than those which ate less freely.

The explanation of these discordant results is not apparent.

Since wide variations in the amount of rice received daily by pigeons did not lead to any regular differences in their susceptibilities to polyneuritis, it was not necessary to ensure by artificial feeding the constancy of the daily rice ration in the quantitative determination of the anti-neuritic values of foodstuffs.

(i) *Meat (lean beef).* (Water content = 75 %.)

Five series of pigeons, *A*, *B*, *C*, *D*, and *E*, each series consisting of five birds, received daily in addition to the rice diet, 2, 4, 6, 10, and 20 gms. of beef respectively.

The results of these experiments are set forth in the following table:

TABLE II. *Meat (raw beef).*

A. Polished rice + 2 gms. beef daily.

	Pigeon 6	Pigeon 7	Pigeon 8	Pigeon 9	Pigeon 10
Effect of diet upon pigeons' health	Weakness in limbs appeared on 22nd day. Acute symptoms of polyneuritis on 27th day.	Acute symptoms of polyneuritis developed on 27th day.	Weakness in limbs appeared on 27th day. Died on 30th day with no acute symptoms of polyneuritis.	Weakness in limbs appeared on 27th day. Died on the 31st day with only slight symptoms of polyneuritis.	Weakness in limbs appeared on 27th day. Symptoms became no more acute. Death on 32nd day.
	Fatty degeneration indicated in the sciatic nerves to various extents.				
Mean change in wt. by end of a certain time	− 20·9 % after 27 days				

B. Polished rice + 4 gms. beef daily.

	Pigeon 11	Pigeon 12	Pigeon 13	Pigeon 14	Pigeon 15
Effect of diet upon pigeons' health	Weakness in limbs on 22nd day. Died. Acute symptoms of polyneuritis developed on 25th day.	Weakness in limbs on 25th day. Died on 26th day without acute symptoms of polyneuritis.	Weakness in limbs appeared on 25th day. Died on 26th day without acute symptoms of polyneuritis.	Acute symptoms of polyneuritis developed on 27th day.	Weakness in limbs appeared on 33rd day. Died on next day without acute symptoms of polyneuritis.
	Fatty degeneration indicated in the sciatic nerves to various extents.				
Mean change in wt. by end of a certain time	− 20·7 % after 20 days				

C. Polished rice + 6 gms. beef daily.

	Pigeon 16	Pigeon 17	Pigeon 18	Pigeon 19	Pigeon 20
Effect of diet upon pigeons' health	Acute symptoms of polyneuritis developed on 27th day.	Weakness in limbs on 27th day. Acute symptoms of polyneuritis developed on 28th day.	Severe weakness in limbs appeared on 32nd day. Died on the same day without acute symptoms of polyneuritis.	Died without symptoms on 33rd day.	Severe weakness appeared in limbs on 34th day. Died on same day without acute symptoms of polyneuritis.
	Fatty degeneration indicated in the sciatic nerves to various extents.				
Mean change in wt. at the end of a certain time	− 16 % after 27 days				

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D. Polished rice + 10 gms. beef daily.

	Pigeon 21	Pigeon 22	Pigeon 23	Pigeon 24	Pigeon 25
Effect of diet upon pigeons' health	Flight impaired on about the 25th day.				
	Weakness in limbs on 32nd day. Died on same day without acute symptoms of polyneuritis.	Weakness in limbs appeared on 32nd day.	Weakness in limbs on 38th day. Died on same day without acute symptoms of polyneuritis.	Weakness in limbs appeared on 32nd day. Died on 34th day without acute symptoms of polyneuritis.	Weakness in limbs and slight symptoms of polyneuritis developed on 59th day. Died on next day.
	Fatty degeneration indicated in the sciatic nerves to various extents.				
Mean change in wt. at the end of a certain time	− 7·4 % after 30 days				

E. Polished rice + 20 gms. beef daily.

	Pigeon 42	Pigeon 43	Pigeon 44	Pigeon 45
Effect of diet upon pigeons' health	Remained quite healthy for 6 weeks. At end of this time ration of meat reduced to 15 gms. After another 28 days typical symptoms of polyneuritis set in.	Died on the 29th day without symptoms.	Remained quite healthy for 6 weeks. At end of this time meat ration reduced to 15 gms. After another 52 days had not developed symptoms of polyneuritis.	Remained quite healthy for 6 weeks. At end of this time meat ration reduced to 15 gms. After another 52 days had not developed symptoms of polyneuritis.
Change in wt. by end of exp.	− 3·9 % (after 1st 6 weeks). − 16·9 % (at end of exp.).	0	+ 15·6 % (after 1st 6 weeks). + 12·5 % (at end of exp.).	− 7·3 % (after 1st 6 weeks). − 8·8 % (at end of exp.).

These results indicate that it was necessary to add 20 gms. of raw lean beef daily to the polished rice diet to prevent the occurrence of symptoms of polyneuritis in pigeons for 50 days. This amount of meat daily was also sufficient to prevent almost entirely the loss in weight which results from an exclusive polished rice diet and in the case of one pigeon it enabled a considerable increase in weight to take place.

The addition of smaller amounts of meat to the rice diet did not prevent the loss in weight and was only sufficient either to delay the occurrence of the symptoms of polyneuritis for a short time or to decrease their severity.

The results of these experiments are of practical importance insomuch as they indicate the small value of meat in the prevention and treatment of beri-beri.

(ii) *Ox-heart.* (Water-content = 60 %.)

Four series of pigeons, *A*, *B*, *C*, and *D*, each series consisting of two birds, received daily in addition to polished rice 5, 10, 15 and 20 gms. of heart-muscle (free from fat) respectively.

The results of these experiments are tabulated below:

TABLE III.

	A. Rice + 5 gms. heart.		B. Rice + 10 gms. heart.	
	No. 189	No. 190	No. 191	No. 192
Effect of diet upon pigeons' health	Quite healthy after 50 days. On 51st day daily ration of heart reduced to 2 gms. Polyneuritis appeared 20 days afterwards.	Quite healthy after 50 days. On 51st day daily ration of heart reduced to 2 gms. Polyneuritis appeared 18 days afterwards.	Quite healthy after 50 days.	Quite healthy after 50 days.
Change in wt. by end of exp.	After 50 days − 3 %. After 70 days − 10 %.	After 50 days − 6·5 %. After 68 days − 16 %.	+ 10·5 %	+ 5·7 %

	C. Rice + 15 gms. heart.		D. Rice + 20 gms. heart.	
	No. 193	No. 194	No. 195	No. 196
Effect of diet upon pigeons' health	Quite healthy after 50 days.	Quite healthy after 50 days.	Quite healthy after 50 days.	Quite healthy after 50 days.
Change in wt. by end of exp.	− 3 %	− 1·4 %	− 6·6 %	− 3 %

The results indicated that the addition of 5 gms. of heart daily to the polished rice diet was quite sufficient to prevent symptoms of polyneuritis for 50 days and also to prevent any appreciable loss in weight.

Since the daily addition of as much as 20 gms. of beef to the rice diet was necessary to prevent polyneuritis for the same period, heart-muscle was considerably superior to voluntary muscle in anti-neuritic power.

The richness of the mammalian heart in the substance preventing polyneuritis may be correlated with the fact that in human beri-beri heart failure accompanied by fatty degeneration is a very common symptom.

(iii) *Sheep-brain.* (Water-content = 80 %.)

Three series of pigeons, *A*, *B*, and *C*, each series consisting of three birds, received daily in addition to the polished rice diet 3, 6, and 15 gms. of sheep-brain respectively.

The results of the experiments are set forth in the following table:

TABLE IV. *Polished rice + Sheep-brains (raw).*

A. Polished rice + 3 gms. brains daily.

	Pigeon 97	Pigeon 98	Pigeon 99
Effect of diet upon pigeons' health	Symptoms of polyneuritis on 21st day.	Symptoms of polyneuritis on 24th day.	Died on 66th day with symptoms of weakness.
Change in weight by end of exp.	− 12 %	− 10 %	0 %

B. Polished rice + 6 gms. brains daily.

	Pigeon 100	Pigeon 101	Pigeon 102
Effect of diet upon pigeons' health	Died on 45th day without symptoms.	Symptoms of polyneuritis on 20th day.	Died on 66th day with symptoms of weakness.
Change in weight by end of exp.	− 3 %	− 3 %	− 12·5 %

C. Polished rice + 15 gms. brains daily.

	Pigeon 103	Pigeon 104	Pigeon 105
Effect of diet upon pigeons' health	Still healthy after 43 days. Daily ration of brain then reduced to 8 gms. Symptoms of polyneuritis 47 days later.	Still healthy after 43 days. Daily ration of brain then reduced to 8 gms. Still healthy after another 47 days.	Still healthy after 43 days. Daily ration of brain then reduced to 8 gms. Still healthy after another 47 days.
Change in weight by end of exp.	End of 1st period (43rd day) − 12·6 %. End of exp. (90th day) − 26·6 %.	End of 1st period (43rd day) + 9·4 %. End of exp. (90th day) − 4·7 %.	End of 1st period (43rd day) − 4 %. End of exp. (90th day) − 21 %.

The experiments indicated that in some cases the daily addition of 3 and 6 gms. of brain to a diet of polished rice delayed the appearance of symptoms of polyneuritis, but to prevent consistently their occurrence for 50 days a daily ration of as much as 12 gms. of brain (the mean of 8 and 15 gms.) was necessary.

Considering the great importance of the anti-neuritic substance in the activities of the nervous system its low concentration in brain is remarkable. This may indicate that in metabolism it is constantly being converted into substances, which do not possess anti-neuritic properties, so that its concentration in the nervous system must always remain small.

The effect of brain-material in maintaining body-weight was very much greater, being demonstrable even when the anti-neuritic substance was present in insufficient amount.

It was previously found that in the case of birds fed exclusively on polished rice considerable loss in weight could only be prevented by giving extra large daily rations.

These results indicate a secondary deficiency in polished rice, namely, that of substances necessary for the maintenance of the body-weight and evidently contained in comparatively large amount in brain. Considerable fatty degeneration was indicated by Marchi's method in the sciatic nerves of the birds which developed polyneuritis without fall in body-weight. The degeneration was usually quite as extensive as that occurring in pigeons in which great loss in weight accompanied the polyneuritis. It would appear that the fatty degeneration is an effect of the deficiency in polished rice of the anti-neuritic substance and not of the secondary deficiency of substances essential for the maintenance of body-weight.

(iv) *Fish.* (Water-content = 80 %.)

Raw hake-muscle was used in the experiments.

Two series of pigeons, each series consisting of four birds, received respectively in addition to the polished rice diet 5 and 10 gms. of fish daily.

The results of these experiments are set forth below:

TABLE V. *Fish* (*Hake*).

Polished rice + 5 gms. fish daily.

	Pigeon 46	Pigeon 47	Pigeon 48	Pigeon 49
Effect of diet upon pigeons' health	Died on 54th day of exp. without symptoms of weakness or polyneuritis.	Developed symptoms of polyneuritis on 20th day.	Developed symptoms of polyneuritis on 30th day.	Developed symptoms of general weakness on 21st day which were followed by acute symptoms of polyneuritis the next day.
Change in wt. by end of exp.	− 22·5 %	− 26·4 %	− 23 %	− 38·3 %

Polished rice + 10 gms. fish daily.

	Pigeon 50	Pigeon 51	Pigeon 52	Pigeon 53
Effect of diet upon pigeons' health	Developed symptoms of general weakness on 21st day which passed to those of polyneuritis on 23rd day.	Developed symptoms of polyneuritis on 23rd day.	Developed symptoms of polyneuritis on 24th day.	Developed symptoms of polyneuritis on 31st day.
Change in wt. by end of exp.	− 23·9 %	− 26·6 %	− 24·6 %	− 28 %

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The results indicated that the addition of as much as 10 gms. of fish daily to the polished rice diet neither delayed the occurrence of polyneuritis nor arrested the fall in weight that results from exclusive rice diets. Fish muscle therefore resembled meat in being deficient in the anti-neuritic substance.

(v) *Egg-yolk.* (Water-content = 50 %.)

Four series of pigeons, *A*, *B*, *C*, and *D*, each series consisting of two birds, received daily in addition to polished rice 1, 2, 5 and 10 gms. respectively of raw egg-yolk.

The effect of cooking upon the anti-neuritic power of egg-yolk was also investigated. The eggs were boiled for 4 minutes and the coagulated yolks were ground with water and administered to the birds by means of a crop-tube.

Two series of pigeons, *E* and *F*, each series consisting of two birds, received daily 3 and 6 gms. respectively of cooked yolk in addition to the rice diet.

The results of the experiments are tabulated below:

TABLE VI. *Polished rice + Raw egg-yolk.*

	A. Pol. rice + 1 gm. yolk daily.		B. Pol. rice + 2 gms. yolk daily.	
	Pigeon 185	Pigeon 186	Pigeon 187	Pigeon 188
Effect of diet upon pigeons' health	Symptoms of polyneuritis on 26th day.	Symptoms of polyneuritis on 12th day.	Symptoms of weakness in limbs on 43rd day. On increasing the daily ration of yolk to 4 gms. the symptoms were prevented from becoming more acute.	Symptoms of weakness in limbs on 41st day. Ration of egg increased to 3 gms. Symptoms had entirely disappeared before 50th day and loss in weight was arrested.
Change in wt. by end of exp.	− 21·9 %	− 17·6 %	By 43rd day − 16·7 %. By 68th day − 28·2 % (end of exp.).	By 41st day − 24·3 %. By 50th day − 21·4 % (end of exp.).

	C. Pol. rice + 5 gms. yolk daily.		D. Pol. rice + 10 gms. yolk daily.	
	Pigeon 136	Pigeon 137	Pigeon 138	Pigeon 139
Effect of diet upon pigeons' health	Still healthy after 55 days.	Still healthy after 55 days.	Still healthy after 55 days.	Still healthy after 55 days.
Change in wt. by end of exp.	− 30·7 %	− 32·1 %	− 7·5 %	− 3 %

	E. Pol. rice + 3 gms. cooked yolk daily.		F. Pol. rice + 6 gms. cooked yolk daily.	
	Pigeon 201	Pigeon 202	Pigeon 203	Pigeon 204
Effect of diet upon pigeons' health	Still healthy after 50 days.	Still healthy after 50 days.	Still healthy after 50 days.	Still healthy after 50 days.
Change in wt. by end of exp.	− 25·7 %	− 24·4 %	− 12·2 %	− 19 %

The daily addition of 5 gms. of raw egg-yolk or of 3 gms. of the cooked yolk to the polished rice diet therefore prevented the occurrence of symptoms of polyneuritis for 50 days and 2 gms. of raw yolk daily delayed the appearance of the symptoms until about the 40th day of the experiment. These disappeared when the ration of egg-yolk was increased to 3 or 4 gms.

The results indicate that the addition of 3 gms. of raw egg-yolk daily to the polished rice would be just sufficient to prevent the occurrence of symptoms of polyneuritis for 50 days.

It is seen that the daily ration of boiled egg-yolk (3 gms.) sufficient for this purpose was about midway between the insufficient daily ration of raw egg-yolk (2 gms.) and the 5 gms. allowance that prevented the occurrence of polyneuritis symptoms for 50 days. It may be concluded from these results that the process of heating at 100° C. for 4 minutes had no measurable effect upon the anti-neuritic value of egg-yolk.

A comparison of the amounts of beef, ox-heart, brain, and egg-yolk, which, when added daily to the rice diet, were sufficient to prevent symptoms of polyneuritis for 50 days, indicates that in the natural condition egg-yolk considerably exceeds the other foodstuffs in its capacity for preventing this disease.

(vi) *Dried brewers' yeast.* (Water-content = 5 %.)

Pressed yeast was sieved and then dried at 37° C. for 2 days. After this treatment it only contained 5 % of moisture. Three series of pigeons, *A*, *B*, and *C*, each series consisting of two or three birds, received daily $2\frac{1}{2}$, 1, and $\frac{1}{2}$ gms. respectively of this dried yeast preparation in addition to polished rice. The results of these experiments are set forth below.

These experiments indicate that dried brewers' yeast possessed considerable preventive power against polyneuritis. One-half a gram of this preparation added to the polished rice diet was more than

sufficient to maintain pigeons free from this disease for 50 days and also to prevent any loss in weight.

As Schaumann (1911) found that the addition of an alcoholic extract of yeast to the polished rice diet, although it prevented polyneuritis, did not prevent the loss in weight, the constituents of yeast that can maintain the body-weight are evidently insoluble in alcohol.

TABLE VII. *Polished rice + Yeast (dried).*

	A. Pol. rice + 2½ gms. dried yeast.		B. Pol. rice + 1 gm. dried yeast.	
	Pigeon 73	Pigeon 74	Pigeon 69	Pigeon 83
Effect of diet upon pigeons' health	At end of 40 days was still healthy.	At end of 40 days was still healthy.	At end of 59 days was still healthy. Daily allowance of yeast then reduced to ¼ gm. and after another 52 days bird was still healthy but had lost in weight.	At end of 54 days was still healthy. After 54 days yeast was stopped and bird received only rice. It developed symptoms of polyneuritis 27 days subsequently.
Change in wt. by end of exp.	+13 %	+1·4 %	After 1st 59 days +12·3 %. At end of exp. −4·1 %.	After 1st 54 days +8·5 %.

C. Pol. rice + ½ gm. dried yeast.

	Pigeon 68	Pigeon 81	Pigeon 82
Effect of diet upon pigeons' health	At end of 59 days was still healthy. Yeast allowance was then reduced to ¼ gm. and bird was still healthy after another 68 days although it had lost in weight.	At end of 54 days was still healthy. Yeast allowance was then stopped and bird developed symptoms of polyneuritis 14 days subsequently and had lost in weight.	At end of 54 days was still healthy. Yeast allowance was then stopped and symptoms of polyneuritis appeared 24 days subsequently and loss in weight occurred.
Change in wt. by end of exp.	After 1st 59 days +6 %. At end of exp. −12 %.	After 1st 54 days +4·7 %. At end of exp. −16·6 %.	After 1st 54 days +5·2 %. At end of exp. −23·7 %.

(vii) *Dried lentils.* (Water-content = 12 %.)

Series of pigeons, *A*, *B*, *C*, and *D*, each series consisting of 2 birds, received daily in addition to polished rice 1, 3, 4½ and 6 gms. respectively of uncooked lentils.

The results of the experiments are tabulated below.

These experiments indicate that the daily addition of 3 gms. of dried lentils to the polished rice diet was sufficient to prevent the occurrence of polyneuritis for at least 50 days, but could not prevent the loss in weight. For the maintenance of the body-weights of the pigeons 6 gms. of lentils daily were necessary.

TABLE VIII. *Polished Rice + Dried Lentils.*

	A. Pol. rice + 1 gm. lentils.		B. Pol. rice + 3 gms. lentils.	
	Pigeon 150	Pigeon 151	Pigeon 106	Pigeon 107
Effect of diet upon pigeons' health	Symptoms of polyneuritis on 38th day.	No symptoms after 39 days.	Healthy after 57 days.	Healthy after 57 days.
Change in weight by end of exp.	− 37 %	− 32 %	− 18 %	− 7 %

	C. Pol. rice + 4½ gms. lentils.		D. Pol. rice + 6 gms. lentils.	
	Pigeon 130	Pigeon 131	Pigeon 108	Pigeon 110
Effect of diet upon pigeons' health	Healthy after 55 days.	Healthy after 55 days.	Healthy after 57 days.	Healthy after 57 days.
Change in weight by end of exp.	− 14·5 %	− 19 %	+ 26 %	+ 18·7 %

(viii) *Unhusked barley.* (Water-content = 12 %.)

Three series of pigeons, each series consisting of 4 birds, received daily by artificial feeding mixed diets of the following compositions:

	Series A	Series B	Series C
Polished rice	15 gms.	12 gms.	8 gms.
Barley	3 ,,	6 ,,	10 ,,
Raw beef	2 ,,	2 ,,	2 ,,

The results of the experiments are set forth in the following table:

TABLE IX.

A. Polished rice 15 gms.; Raw beef 2 gms.; Unhusked barley 3 gms. daily.

	Pigeon 54	Pigeon 55	Pigeon 56	Pigeon 57
Effect of diet upon pigeons' health	Symptoms of polyneuritis on 17th day.	Still healthy after 57 days but had lost 17·7 % in weight. Diet altered on 57th day thus: Pol. rice 13 gms., Beef 2 gms., Barley 5 gms. This led to rise in weight and after 33 days more final change in weight was − 4·1 %.	Weakness in limbs on 18th day which passed to acute symptoms of polyneuritis on 26th day.	Weakness in limbs on 57th day with loss in weight of 33·3 %. On 57th day diet was altered thus: Pol. rice 13 gms., Beef 2 gms., Barley 5 gms. This led to complete disappearance of weakness and to rise in weight, the final loss after 33 days more being 25·6 %.
Change in wt. by end of exp.	− 19·7 %	...	− 14·3 %	...

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B. Polished rice 12 gms.; Raw beef 2 gms.; Unhusked barley 6 gms. daily.

	Pigeon 58	Pigeon 59	Pigeon 60	Pigeon 61
Effect of diet upon pigeons' health	After 53 days was still healthy.	After 53 days was still healthy.	Died on the 5th day without symptoms.	After 53 days was still healthy.
Change in wt. at end of exp.	0	−13·8 %	0	+30·6 %

C. Polished rice 8 gms.; Raw beef 2 gms.; Unhusked barley 10 gms. daily.

	Pigeon 62	Pigeon 63	Pigeon 64	Pigeon 65
Effect of diet upon pigeons' health	After 53 days was still healthy.	Died on 11th day without symptoms.	After 53 days was still healthy.	After 53 days was still healthy.
Change in wt. at end of exp.	0	0	−9·9 %	+1·3 %

These experiments indicate that birds fed on a mixed diet consisting of polished rice 12 gms., meat 2 gms., barley 6 gms., did not develop symptoms of polyneuritis in 53 days and that a diet containing polished rice 13 gms., meat 2 gms., barley 5 gms., could recuperate birds in the early stages of polyneuritis resulting from diets consisting too exclusively of polished rice.

Since a daily ration of 2 gms. of meat scarcely delayed the symptoms of polyneuritis, it is probable that the addition of 5 gms. of barley to the rice diet daily is sufficient to prevent the occurrence of polyneuritis in pigeons for 50 days. Takaki (1885–87) by the addition of meat and barley to the polished rice diet was able to eradicate almost completely beri-beri from the Japanese Navy. A comparison of the anti-neuritic values of barley and meat indicates that the prevention of the disease was due to the former to a greater extent than to the latter.

The proportion of barley in the above mixed diets was also found to influence the body-weights of the birds. While the pigeons receiving only 3 gms. of barley in their daily ration all lost considerably in weight, of those receiving 6 and 10 gms. of barley daily with correspondingly smaller rations of polished rice, some lost to a small extent, some gained and others remained stationary in weight.

These results indicated that a total ration of 20 gms. of grain containing 10 to 12 % of water provided it contained a certain proportion of barley were quite sufficient to keep adult pigeons weighing about 350 gms. in nutritive equilibrium. The loss in weight that accompanies polyneuritis induced by rations of polished rice equal to the above in bulk does not appear to be due to an inadequate supply of protein or carbohydrate[1], but to a deficiency in polished rice of certain substances of at present unknown nature.

[1] During the process of polishing rice retains its carbohydrate and nearly all the protein, but becomes deficient in fat and phosphorus.

Osborne and Mendel (1911) have shewn that, while both young and adult rats could be maintained in body-weight on diets consisting of pure proteins, carbohydrates, lard and salts, there was no indication of growth in the former. When milk or extracts of milk free from protein and fat, however, were added to the dietaries, growth was found to proceed. Hopkins (1912) has obtained similar results with young rats. It would appear that the substances essential for growth and for maintenance of body-weight are not identical.

A comparison of the preventive powers of the various foodstuffs against polyneuritis and loss in weight.

In the following table are compared the amounts of various uncooked foodstuffs required to be added daily to the polished rice diet to prevent firstly, the occurrence of symptoms of polyneuritis in pigeons of an average weight of 350 gms. for fifty days, and secondly, any appreciable loss in weight.

Foodstuffs	Daily ration required to prevent polyneuritis		Daily ration required to prevent loss in weight	
	In terms of natural foodstuff	In terms of dry-weight	In terms of natural foodstuff	In terms of dry-weight
Beef	20 gms.	5 gms.	20 gms.	5 gms.
Ox-heart ...	5	2	5	2
Sheep-brain ...	12	2·5	3 to 6	0·6 to 1·2
Fish (Hake) ...	>10	>2	>10	>2
Egg-yolk ...	3	1·5	10	5
Yeast (pressed) ...	2·5	0·5	2·5	0·5
Lentils ...	15	3	30	6
Barley {Unhusked	3·75	3·25	7·5	6·5
Barley {Husked...	5	4·5	10	9

The results indicate that in the natural condition yeast and egg-yolk were nearly equal in anti-neuritic value and greatly exceeded the other foodstuffs in this respect. On comparing the preventive powers in terms of dry weight, however, yeast was three times as efficient as egg-yolk.

Of the foodstuffs examined of animal origin heart-muscle and egg-yolk were the most efficient, while beef was of particularly small anti-neuritic value and, in fact, was the most inefficient of the above eight foods with the possible exception of fish. On comparing the vegetable substances yeast was found to be more potent in preventing neuritis than a dried pulse (lentils) and a husked cereal (barley), which were about equal in anti-neuritic value.

A consideration of the above table shews that the amounts of the various foodstuffs required to be added to the polished rice diet to prevent polyneuritis and to maintain the body-weight did not go hand in hand. Thus a potent anti-neuritic substance was not necessarily efficient in preventing loss in weight.

For convenience the foods can be arranged into three classes according to their relative efficiencies in preventing neuritis and in maintaining body-weight.

Class I. Foodstuffs *inefficient* in both capacities.

Polished rice, beef.

Class II. Foodstuffs *efficient* in both capacities.

Yeast, heart, brain.

Class III. Foodstuffs efficient in preventing polyneuritis but less efficient *in maintaining* body-weight.

Egg-yolk, barley, lentils.

It is concluded that the capacities of the various foodstuffs for preventing polyneuritis and maintaining body-weight are due to distinct constituents. The substances possessing the latter property are probably not proteins in general, since beef is so inefficient in preventing the loss in weight induced by the rice diet. They appear also not to be fats or lipoids, because egg-yolk which is very rich in these substances is not efficient in preventing the loss in weight. Further, polished rice is so deficient in fat (0·2 %) that its ability to prevent a great loss in weight and even to maintain the body-weight when given in *large ration* cannot be ascribed to this constituent.

As already stated Schaumann (1911) shewed that, although they are not affected with polyneuritis, birds lose weight considerably when alcoholic extract of yeast is added to the polished rice diet. With the addition of an equivalent amount of the original yeast, however, the birds are quite healthy and body-weight is maintained. He considers that the anti-neuritic substance is an activator which renders possible the assimilation of phosphorus by the organism. He further supposes that the yeast acts not only by virtue of its content of this substance, but also through its constituent phosphorus, which supplements the phosphorus-content of the rice, thus effecting a maintenance of the body-weight, and that the inability of the alcoholic extract to prevent loss in weight is due to the fact that the phosphorus-content of yeast is only partially extracted by alcohol.

As it is possible however for polyneuritis to occur even when the body-weight is maintained it would appear that the polyneuritis and

loss in weight induced by diets of polished rice are due to distinct causes, the former to a deficiency in the anti-neuritic substance and the latter to a deficiency of one or more substances of at present unknown nature.

A comparison of the anti-neuritic values of the above foodstuffs indicates that egg-yolk, ox-heart, yeast, lentils and barley will be effective in the prevention of beri-beri. Lentils and barley on account of their cheapness and suitability will probably be found the most practicable for this purpose.

Part II.

The curative properties of extracts of various foodstuffs towards polyneuritis in pigeons.

The various extracts were given orally to the pigeons, exhibiting the acute symptoms of polyneuritis. At the time of receiving the doses the condition of the birds was such that untreated they would have died within 24 hours. There was often a marked improvement two or three hours after the administration of a curative extract and recovery was generally complete within 24 hours.

(*a*) *Meat* (*Beef*).

Beef juice. Raw beef was well minced and pressed by means of a screw-press. The extract was then concentrated *in vacuo* at 30° C.

In another experiment the minced beef was thoroughly mixed with sand and Kieselguhr, the mass pressed in the Buchner press and the extract concentrated *in vacuo* at 30° C.

These extracts caused no improvement in the condition of four pigeons when administered in amounts equivalent to from 3 oz. to 1½ lbs. of the original meat.

The alcoholic extract. Thoroughly minced beef was shaken for three hours with an equal weight of absolute alcohol in the shaking machine; the mixture was then pressed by means of the screw-press and the extract concentrated *in vacuo* at 30° C. Taking into consideration the high water-content of meat, the extraction was in reality carried out with 60% alcohol (approx.). Doses of the concentrated preparation equivalent to amounts of beef varying from 4·6 to 6·4 oz. were sufficient to cause the complete recovery of three birds affected with polyneuritis. Some of the concentrated alcoholic extract was extracted at ordinary

temperatures with purified ether. The ethereal layer was separated and concentrated at 20° C. *in vacuo*. The residue of the alcoholic extract insoluble in ether was also evaporated *in vacuo* to remove all dissolved ether.

The ethereal extract was administered to four birds affected with polyneuritis in amounts equivalent to and even much greater than the curing dose of the original alcoholic extract and was found to possess no curative properties.

The residue of the alcoholic extract was given to a pigeon in an amount equivalent to the curing dose of the original alcoholic extract and the bird completely recovered in 24 hours except that its flight was still slightly weak.

In another experiment the meat was first of all dried at room temperature by means of the electric fan and it was then ground and extracted with absolute alcohol in the shaking machine. The filtered extract was concentrated *in vacuo* at 30° C. to remove all the alcohol. An amount of this extract equivalent to 8 oz. of meat effected the complete recovery of a bird ill with polyneuritis.

A portion of this alcoholic extract was shaken with 10 times its volume of water; the liquid was filtered and the turbid filtrate shaken with a little ether. This caused the liquid to separate into three layers, (i) A top ethereal layer, containing only a trace of solid matter which was of a fatty nature, (ii) a middle layer of a turbid character, (iii) a third clear aqueous layer.

The curing dose of the bottom aqueous layer was about equivalent to that of the original alcoholic extract.

A dose of the turbid layer equivalent to the curing dose of the original alcoholic extract caused a slight improvement in the condition of another bird, but a relapse speedily set in followed by death. The residue of the alcoholic extract insoluble in water when administered in an amount equivalent to twice the curing dose of the original alcoholic extract greatly improved the condition of two pigeons, but did not effect complete recovery, as three days later the birds were still lame and weak in flight and shortly afterwards they died. These results indicate that the curative constituent was almost entirely removed from the alcoholic extract by water. Since this substance could not be extracted by water from the original meat, it must be set free in some way during the alcoholic extraction.

The ethereal extract. The meat dried in the way already described was extracted with purified ether; the extract was filtered and the

ether evaporated off at 20° C. *in vacuo*. This extract possessed curative properties against polyneuritis, but it was considerably less potent than the alcoholic extracts, since 11 gms., an amount equivalent to about one kilogram of undried meat, although it caused a marked improvement in eight hours, did not entirely remove the lameness of the bird for six days and even then the power of flight was not entirely regained.

Summary. The constituent of meat that cures polyneuritis is therefore not extracted by water and only to a small extent by ether. It is readily removed from meat by both 60 % and absolute alcohol and after the alcoholic extraction is almost completely soluble in water. This suggests that it is in some way set free during the process.

(*b*) *Egg-yolk.*

Twenty-four egg-yolks, weighing 350 gms., were extracted with 1700 c.c. of absolute alcohol in the shaking machine at ordinary temperatures. Taking into consideration the water-content of the yolk (50 %), this was equivalent to extraction with about 90 % alcohol. The extract was filtered and the alcohol removed by evaporation *in vacuo* at 30° C.

An amount of this extract equivalent to four egg-yolks completely cured a pigeon affected with polyneuritis, while a dose equivalent to seven yolks given to another pigeon greatly improved its condition but could not remove the lameness.

In a later experiment twenty egg-yolks weighing 300 gms. were dried by means of the electric fan at 20° C. and the dry ground yolk was thoroughly extracted with purified ether in the shaking machine. The ether was evaporated from the filtered extract at 20° C. *in vacuo*.

Eight gms. of the dried ethereal extract (= 3 egg-yolks) were sufficient to cure a pigeon affected with polyneuritis and to prevent the reappearance of the symptoms for 14 days. Five gms. and 7 gms. of this ethereal extract improved the conditions of pigeons in 3 to 14 hours, but could not entirely remove the lameness and flight weakness. Nevertheless these amounts were sufficient to delay the reappearance of the acute symptoms of polyneuritis for about ten days.

The residue of the egg-yolks insoluble in ether was next extracted with absolute alcohol and the filtered extract was concentrated *in vacuo* at 30° C. An amount of this extract equivalent to ten egg-yolks cured a pigeon of polyneuritis and the symptoms did not reappear until eight days afterwards.

Summary. The constituent of egg-yolk that cures polyneuritis in pigeons is therefore readily extractable with 90% alcohol and with ether; after extraction with the latter a further yield can be obtained from the residue by means of absolute alcohol.

(*c*) *Dried lentils.*

The lentils were thoroughly ground in a coffee-mill and one kilogram was extracted at 20° C. in the shaking machine with three litres of absolute alcohol. The mixture was filtered and the residue again extracted with three litres of alcohol. The combined filtrate was concentrated *in vacuo* at 30° C. to remove all the alcohol. The dried extract, which was a yellowish-red fatty mass, weighing 30 gms. and an amount of it equivalent to 20 gms. of lentils was just sufficient to bring about the complete recovery of pigeons affected with polyneuritis.

A portion of the alcoholic extract was shaken at 40° C. with ten times its volume of water. The filtered extract was concentrated *in vacuo* at 30° C. and the concentrated liquor was found to possess curative properties.

To this aqueous extract a solution of basic lead acetate was gradually added, until there was no more precipitation and the mixture was allowed to stand six hours and was then filtered. The filtrate was a dark yellow liquid with a somewhat ammoniacal odour. The traces of lead were removed from it by careful precipitation with sulphuric acid and the filtrate was concentrated *in vacuo* at 30° C. in the presence of a very slight excess of sulphuric acid. The curing dose of the concentrated filtrate was approximately equivalent to that of the original alcoholic extract. This indicates that the anti-neuritic constituent is not precipitated by basic lead acetate.

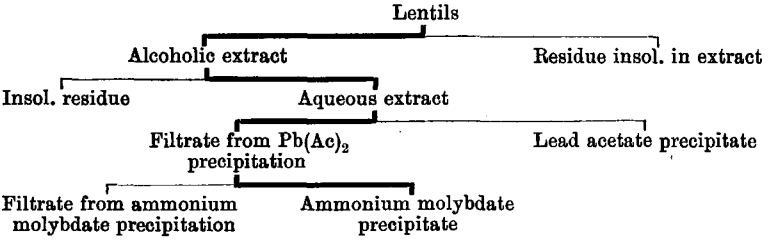
The filtrate gave no precipitate with zinc sulphate, cadmium nitrate, cobalt sulphate, uranium acetate and tannic acid, but gave a slight precipitate of a blue colour with copper sulphate, a copious white precipitate with silver nitrate and also with acetone. Ammonium molybdate yielded a voluminous yellow precipitate. To some of this filtrate a solution of ammonium molybdate was gradually added until there was no further precipitation. The mixture was allowed to stand for 12 hours and was then filtered; the precipitate was washed with water, shaken for two hours with an aqueous suspension of baryta, filtered, and the barium was removed from the filtrate with sulphuric acid. The filtrate free from baryta was concentrated *in vacuo* at 30° C. in the

presence of a trace of sulphuric acid. An amount of the concentrated liquor equivalent to 100 gms. of lentils improved the condition of a bird affected with polyneuritis in three hours, but did not effect a complete recovery, as the lameness and weakness in flight had not disappeared in 48 hours and acute symptoms of polyneuritis reappeared at the end of this period. A dose equivalent to 250 gms. of lentils, however, brought about the complete recovery of another pigeon which did not develop symptoms of polyneuritis again until nine days afterwards.

The filtrate from the ammonium molybdate precipitation was carefully treated with baryta to precipitate the excess of molybdate and, after the removal of the precipitated barium molybdate by filtration, was concentrated *in vacuo* at 30° C. in the presence of a little sulphuric acid. An amount of the concentrated filtrate equivalent to 100 gms. of lentils caused no improvement in a pigeon ill with polyneuritis; an amount equivalent to 250 gms., however, possessed very slight curative properties.

These facts show that the curing constituent of lentils is almost completely precipitated by ammonium molybdate. The curative dose of the decomposed precipitate, however, was very much larger than that of the filtrate after the lead acetate fractionation. This points to the disappearance of some of the active substance during the succeeding fractionation (with molybdate). Possibly the substance was destroyed by contact with the alkali.

Summary of process of fractionation of lentils.



N.B. The thick line indicates the *main* course of the curative constituents during this fractionation.

The action of strychnine upon pigeons affected with polyneuritis.

Pigeons affected with polyneuritis to such a degree that untreated they would have died within 24 hours received orally doses of strychnine varying from 0·0002 gm. to 0·025 gm. The strychnine was found to possess no curative properties, but, although the symptoms of polyneuritis

remained as acute as ever, it was able to prolong the life of the birds for periods varying from two to five days. Strychnine thus appears to resemble in physiological action a crystalline body obtained by Funk (1912) from lime-juice by fractionation with silver nitrate, which was able to keep birds affected with polyneuritis alive for 72 hours without improving their condition.

It is interesting to note in this connection that daily injections of strychnine are stated to have been successful in the treatment of beri-beri.

Summary.

I. *On the distribution of the anti-neuritic substance amongst foodstuffs.*

1. The amounts of various raw foodstuffs that must be added to the polished rice diet to prevent symptoms of polyneuritis in pigeons for a definite period have been determined. The results indicate that the anti-neuritic body is most irregularly distributed amongst foodstuffs.

2. Pigeons fed naturally on daily rations of polished rice of $\frac{1}{20}$th the body-weight for the first two weeks and on much smaller rations subsequently develop symptoms of polyneuritis three to four weeks after being placed on this diet and lose from 10 to 40 % in weight.

3. Pigeons fed on daily rations of polished rice of from $\frac{1}{11}$th to $\frac{1}{30}$th the body-weight develop symptoms of polyneuritis in a much shorter time (in about 14 days). The explanation of this may be that birds fed naturally on small insufficient rations have to rely on their own tissues for supplementing the food supply and are thus able to obtain for use a larger proportion of the anti-neuritic substance distributed in the tissues than the well-fed birds. Consequently the former remain free from polyneuritis for a longer period than the latter.

4. As much as 20 gms. of raw beef are necessary daily to prevent polyneuritis in pigeons weighing about 350 gms. The small anti-neuritic value of this foodstuff indicates that the addition of flesh to a diet is not likely to be very effective in preventing beri-beri.

5. The heart-muscle of the ox greatly exceeds the voluntary muscle of the same animal in its capacity for preventing polyneuritis. This is interesting as in human beri-beri heart failure is a common symptom.

6. Sheep-brain is only about twice as efficient as beef in preventing polyneuritis. The low concentration of the anti-neuritic substance in brain suggests that the substance although essential for the integrity of the nervous tissues is not contained in them as such.

7. Although the addition of small amounts of brain to a diet of polished rice does not prevent polyneuritis, it is effective in almost entirely checking the loss in weight that ensues on this diet. Since birds fed exclusively on polished rice can only be maintained in body-weight by giving very large rations, it is concluded that there is a secondary deficiency in polished rice of substances essential for the maintenance of body-weight and that these are comparatively abundant in brain.

8. Polyneuritis is accompanied by fatty degeneration in the nervous system whether body-weight is maintained or not. This indicates that the degeneration is an effect of the deficiency in polished rice of the anti-neuritic substance and not of the secondary deficiency of substances necessary for the maintenance of body-weight.

9. The daily addition of 10 gms. of fish (hake) to the rice diet did not prevent polyneuritis and loss in weight.

10. Egg-yolk exceeds all the other foodstuffs examined of animal origin in anti-neuritic value, three grams daily added to the rice diet being sufficient to prevent polyneuritis. Its capacity for preventing this disease is not measurably altered by boiling for four minutes.

11. Dried lentils and unhusked barley are efficient anti-neuritic foodstuffs. Three and five grams respectively of these substances added daily to the rice diet are sufficient to prevent polyneuritis.

12. The loss in weight induced by daily rations of polished rice equivalent to about $\frac{1}{20}$th the body-weight can be prevented without altering the actual quantity of food given by substituting barley for polished rice to the extent of about 30 %. This loss in weight is therefore shewn to be due to a genuine deficiency in polished rice and is not the effect of underfeeding.

13. One-half a gram of dried yeast daily is quite sufficient to prevent polyneuritis and also to maintain the body-weights of birds fed on polished rice.

Yeast is more efficient in preventing this disease than any of the above foodstuffs.

14. Egg-yolk, heart-muscle, yeast, lentils and barley will be effective in the prevention of beri-beri. On account of their cheapness lentils and barley will probably be the most practicable for this purpose.

15. The relative efficiencies of the various foodstuffs in preventing polyneuritis and in maintaining the body-weights of birds fed on

polished rice do not correspond. This indicates that the anti-neuritic constituent and the substances maintaining body-weight are not identical. Evidence is brought forward in the text which seems to indicate that the latter substances are not protein, fatty or lipoidal in nature.

II. *On the curative properties of extracts of various foodstuffs.*

1. *Meat.* The anti-neuritic constituent is not extracted by water and only to a small extent by ether. It is readily extracted by alcohol and after this procedure, although still insoluble in ether, is easily dissolved by water.

2. *Egg-yolk.* The curative constituent is present in a form readily extracted by both alcohol and ether. It is not however entirely removed by the latter solvent, since an alcoholic extract of egg-yolk which has been thoroughly exhausted with ether still possesses marked curative properties. These results indicate that a large portion of the anti-neuritic substances contained in meat and egg-yolk is not combined with the fats or free (ether-soluble) lipoids, but is possibly present in the *combined* lipoids, which are only extracted by alcohol.

3. *Lentils.* The constituent of lentils that cures polyneuritis is soluble in strong alcohol and in water, is not precipitated by basic lead acetate and is almost entirely precipitated by ammonium molybdate. There is evidence that the active substance disappears in large quantities during the latter fractionation. It is possibly destroyed by contact with alkali.

Strychnine. Strychnine does not cure polyneuritis but prolongs the lives of pigeons affected with this disease.

I am glad to have this opportunity of expressing my best thanks to Dr C. J. Martin, F.R.S., for much helpful advice in the course of this investigation.

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