THE INFLUENCE OF METABOLIC FACTORS IN BERI-BERI. PART I. THE EFFECT OF INCREASING THE CARBOHYDRATE RATION ON THE DEVELOPMENT OF POLYNEURITIS IN BIRDS FED ON POLISHED RICE.

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It is now taken as definitely settled that the disease beri-beri is essentially privatory in origin, resulting from the deficiency in the food supply of a substance or substances the precise nature of which is at present unknown. If this simple explanation were complete it would follow that the more rapid and thorough the withdrawal of the active substance, the speedier would be the onset of the disease and the more severe its effects.

Maurer (1907) found, however, that when fowls were fed on polished rice those birds receiving the largest quantity of rice were the first to develop symptoms of polyneuritis and that by feeding small amounts of rice the disease could be postponed for a long time.

Chamberlain, Bloombergh, and Kilbourne (1911) on the other hand found that birds consuming large rations of polished rice remained free from polyneuritis for a longer time than birds eating little or no rice at all.

Cooper (1913) however obtained results similar to Maurer, finding that pigeons eating very small amounts of polished rice developed symptoms of polyneuritis in from 20 to 33 days, while those birds receiving daily rations of rice exceeding 1/30th their initial body-weight fell ill in from 9 to 26 days. No constant difference however was noticed in the periods elapsing before the appearance of polyneuritis in the

case of the pigeons receiving daily rations of polished rice varying from 1/11th to 1/30th the initial body-weight.

Caspari and Moszkowski (1913) made observations of a similar nature. They found that pigeons fed on hens' eggs, grape-sugar, and salt remained quite healthy and increased in weight, but pigeons fed on the same rations of egg, sugar, and salt with the addition daily of polished rice developed polyneuritis.

Funk (1914) has still more recently obtained similar results to Maurer (1907) and Cooper (1913), having found that birds fed on large rations of polished rice develop polyneuritis in a shorter time than birds fed on small rations. He also fed series of pigeons on dietaries containing respectively excess of starch, sugar, protein, and fat, and found that the birds receiving the large rations of starch were the first, while those receiving excess of fat were the last, to develop polyneuritis, the birds fed on large rations of sugar and protein falling ill intermediately.

It is thus seen that when the food ration is increased by the addition of material, *e.g.* polished rice, with a very low content of anti-neuritic substance, although the daily allowance of active substance is slightly increased, the development of polyneuritis is actually accelerated.

Similarly birds deprived of everything but water and therefore receiving no anti-neuritic substance at all may live longer than birds fed on a diet, *e.g.* polished rice with a very low content of the active substance, and may die finally without any indication of polyneuritis, although occasional cases of polyneuritis with fatty nervous degeneration were observed by Chamberlain, Bloombergh, and Kilbourne (1911) in fowls. The animal supplied with a little of the substance thus succumbs to the disease, but that receiving none at all may fail to do so. It has been suggested that the starved animal drawing upon its own tissues for a supply of combustible material obtains an adequate amount of anti-neuritic substance in the process of tissue destruction. This is a plausible explanation of the phenomenon, but it does not account for the results of Experiments VI, X and XI (in the present paper) which show first that birds fed on rations equal to 1/20th of their body-weight of washed polished rice lost as much as 40 per cent. in weight and yet developed polyneuritis, and, secondly, that a supply of anti-neuritic substance adequate for the organism provided that the carbohydrate ration was maintained at a certain magnitude was unable to avert polyneuritis when the carbohydrate ration was increased.

Certain facts concerning human beri-beri are parallel to these experimental observations made in the bird. Thus paradoxically, it has been

found by nearly all observers (*Cause and Prevention of Beri-Beri*, Braddon, 1907, p. 256) that in epidemics of beri-beri the well nourished are the first to succumb and are actually more liable to the disease than the underfed. Similarly, under natural conditions men, owing to their larger energy output, partake of polished rice much more freely than women and are more liable to beri-beri. In institutions however where men and women have the same fixed rations the sexes are equally susceptible to the disease. Again, in prisons and asylums in which the dietary has been varied from time to time, every increase in the polished rice component of the diet, the rations of other foodstuffs remaining the same, was attended by increased beri-beri. From these observations Braddon drew the conclusion that in rice-eating communities the extent and severity of beri-beri vary directly with the quantity, absolute or relative, of polished rice consumed.

Admitting, then, that in the production of beri-beri deprivation of a certain substance is an essential factor, it is of obvious importance to ascertain the precise *rôle* which the active substance plays in metabolism, and to determine, if possible, the quantitative relations which the intake of it must bear to the other components of the dietary, *i.e.* to obtain some information as to the composition of diets best adapted for promoting, first, a good absorption, and, secondly, an economical utilization of the active substance and yet adequate for maintaining the organism in nutritive equilibrium.

With this end in view and also on account of its special importance in the prevention of beri-beri the effect upon the development of polyneuritis in birds of increasing the carbohydrate ration of a dietary containing a certain amount of the anti-neuritic substance has been studied quantitatively and the results obtained are set forth in this communication.

Experiments I to XII were carried out by one of us (W. L. B.) at Seremban, Federated Malay States, during the years 1909–11 and the results are borne out by the remaining experiments which were undertaken by the other of us (E. A. C.) at the Lister Institute during 1913.

I. *The effect of rice-diets upon the health and body-weight of birds.*

Experiment I. Padi as an exclusive food for fowls.

Padi, the whole unhusked rice grain, containing at the best about seven per cent. of protein and one per cent. of fat, is by no means a good food for birds, but it was found that fowls fed daily on 1/20th their body-weight of padi could be maintained in fair health for from 60 to 100 days,

after which they usually died without however any indication of polyneuritis, although some had lost considerably in weight.

TABLE I.

Bird	Change in weight by 30th day
1	−20 per cent.
2	+18 ,,
3	−37 ,,
4	+10 ,,
5	−23 ,,
6	−23 ,,
7	−24 ,,
8	+27 ,,
9	− 7 ,,
10	− 7 ,,

Experiment II. Cured or unpolished rice.

Cured or parboiled unpolished rice contains all the elements of the original padi except the husk and coloured layer of the pericarp, which are rubbed off together after the process of boiling or steaming and subsequent drying. This rice is about 10 per cent. richer in protein and fat than padi.

Four fowls fed on 1/20th their body-weight of this rice remained free from polyneuritis, but died on the 38th to 78th day of the experiment of intercurrent disorders.

Six birds fed on rations of "cured" rice varying from 1/10th to 1/26th initial body-weight with two exceptions maintained their weight for four weeks as indicated below.

TABLE II.

Bird	Ration	Change in weight by 26th day
85	1/26th body-weight	0
86	1/13th ,,	−17 per cent.
87	1/10th ,,	0
88	1/16th ,,	0
89	1/15th ,,	−17 per cent.
90	1/12th ,,	0

Experiment III. "Fresh" rice.

Fresh rice, or partly polished hand-pounded rice prepared by the Malay natives for home use, retains portions of the inner pericarpal layer or silver skin, as well as much of the aleurone layer which contains about half the total proteids and fats of the seeds. Hence the rice

has a food value almost as good as that of padi or parboiled rice. Fowls fed naturally on this rice, *i.e.* on rations less than 1/20th body-weight, maintained body-weight and remained healthy for at least one month, as shown in the following table.

TABLE III.

Bird	Change in weight by 30th day
55	0
56	+35 per cent.
57	0
58	0
59	−40 per cent. (Died, but no polyneuritis)
60	0

The rice, such as was used in the above experiments, in which the surface layer of the endosperm is wholly preserved, contains on an average 7 per cent. of protein and 80 per cent. of carbohydrate. Roughly it may be said therefore that physiological balance could be maintained on a diet of which protein composed about 1/300th and carbohydrate 1/25th the body-weight of the birds when sufficient anti-neuritic substance was provided.

The following experiments illustrate what happens, first, when the supply of anti-neuritic substance is reduced, the other food materials remaining constant, and, secondly, when the intake of active substance is kept constant and the carbohydrate ration increased.

Experiment IV. Polished Siam Rice, 1/20th body-weight.

Fowls fed on the above ration developed polyneuritis in from 24 to 69 days, six birds falling ill on the 24th, 25th, 42nd, 43rd, 47th, and 69th days respectively.

It is seen that this rice induced polyneuritis somewhat slowly, but a comparison of the results with those obtained in Experiments I to III indicates that its content of anti-neuritic substance was much smaller than that of padi, cured, or fresh rice.

Experiment V. Washed polished Siam rice, 1/20th body-weight.

In this experiment the rice was first soaked in excess of water for 24 hours. Fowls fed on the above rations developed polyneuritis in from 10 to 35 days, six birds falling ill on the 10th, 13th, 14th, 24th, 32nd, and 35th days respectively. It is seen that this rice induced

polyneuritis more rapidly than the original polished rice, indicating that it had been to a considerable degree deprived of active material by the process of soaking.

Experiment VI. Washed polished Siam rice, 1/20th body-weight.

In this experiment the rice was soaked in excess of water for 48 hours before feeding, and fowls fed on the usual ration developed polyneuritis in 15 to 17 days (15, 15, 15, 15, 16, 17). By the prolonged washing the content of anti-neuritic substance was thus still further diminished if not wholly cut off, and polyneuritis was greatly accelerated. The loss in weight in the case of these birds amounted to as much as 25 to 40 per cent. at the end of the fortnight and in general exceeded anything observed in the other series as shown in the following table. This indicates apparently that the changes culminating in the final break-down (polyneuritis) begin immediately with the deprivation of the protective substance.

TABLE IV.

Bird	Change in weight at end of 14 days			
	Padi 1/20th body-weight ration	Cured rice 1/10th—1/26th	Fresh rice (less 1/20th body-weight) (partly-polished)	Siam pol. rice washed 48 hrs. 1/20th body-weight
1	−30 %	+ 4 %	0	−40 %
2	0	− 4	+20 %	−36
3	−35	− 5	+ 5	−23
4	0	− 6	0	−25
5	−23	−14	−20	−40
6	−30	− 5	+ 3	−35
7	−26			
8	−10			
9	− 7			
10	−10			

The following experiment corroborates these results and also illustrates a point of great practical importance.

Experiment VII.

Ten fowls were fed on 1/20th their body-weight of cured or parboiled rice which had been previously soaked in cold water for 24 hours. Nine of the birds developed polyneuritis in from 13 to 39 days as will be shown in Table V, although birds fed on the same ration of the original parboiled rice had not developed polyneuritis on the 38th to 78th days.

The custom of soaking rice before cooking and then discarding the water has been practised in some institutions and has actually been accountable for severe epidemics of beri-beri amongst the inmates.

In the case of two of the 10 fowls fed on the soaked parboiled rice post-mortem examination revealed general oedema, hydropericardium, and distension of gall bladder, appearances rarely occurring in avian beri-beri. This suggests that further experiments with this rice may lead to the differentiation of the factor which determines the occurrence of oedema in human beri-beri.

The effects of the various rices upon the health of birds are classified in the following table.

TABLE V.

Rations 1/20th body-weight.

				Polished Siam rice			
				1	2	3	
No.	Padi	Cured Rice (unpolished rice)	Fresh Rice (partly polished by hand)	Original	Soaked in excess of water 24 hrs.	Soaked for 48 hrs.	Cured rice Soaked for 24 hrs.
				Polyneuritis appeared on			
1	Died on 67th day. No polyneuritis	Died on 38th day. No polyneuritis	All healthy after 4 weeks	24th day	10th day	15th day	13th day
2	Died on 72nd day. No polyneuritis	Died on 47th day. No polyneuritis		25th ,,	13th ,,	15th ,,	15th ,,
3	Died on 93rd day. No polyneuritis	Died on 54th day. No polyneuritis	...	47th ,,	14th ,,	16th ,,	18th ,,
4	Died on 96th day. No polyneuritis	Died on 78th day. No polyneuritis	...	42nd ,,	24th ,,	15th ,,	26th ,,
5	Still healthy on 100th day	...	...	43rd ,,	32nd ,,	16th ,,	32nd ,,
6	...	...	...	69th ,,	35th ,,	17th ,,	39th ,,
7	...	...	...	...	...	...	16th ,,
8	...	...	...	...	...	...	23rd ,,
9	...	...	...	...	...	...	25th ,,
10	...	...	...	...	...	...	26th ,,
11	...	...	...	...	...	...	34th ,,
12	...	...	...	...	...	...	Died on 63rd day without polyneuritis

Experiments IV to VII prove no more than is well known that the consumption of rice deprived of a certain substance induces beri-beri. The observations however have value according to the light in which they are regarded. In Experiments I to III the birds were fed on a ration containing a definite amount of starch and a definite amount of

anti-neuritic substance, the association of which with the starch prevented polyneuritis. In Experiments IV to VII it is seen that the ration of carbohydrate remaining constant, while the supply of active substance was reduced or withdrawn, beri-beri followed and the effect varied directly with the extent of withdrawal.

The first three experiments furnish a rough index of the normal quantity of anti-neuritic substance necessary daily to maintain physiological balance in a fowl when a normal ration of food is ingested. This quantity is the amount contained in a bulk of padi equal to 1/20th the body-weight of the bird.

Experiments were next made with commercial starch such as is sold for laundry purposes. 10 gms. daily were given alone to fowls of 300 to 500 gms. and the results are tabulated below.

Experiment VIII. Five fowls fed on a ration of 10 *gms. of pure starch only, daily.*

TABLE VI.

No.	Day, and weight in grammes											Results
	1	4	6	8	10	12	14	16	18	20	24	
37	525	510	570	600	540	480	433	450	360	375	345	Became weak on 16th day and remained so till 20th but no signs of beri-beri. Food changed to padi but died 24th day.
49	375	375	405	360	240	—	—	—	—	—	—	Sickened but no signs of paralysis, died 10th day.
50	300	270	365	300	300	240	225	173	—	—	—	Gradually wasted, on 14th day very weak but no paralysis. Died 16th day.
51	480	435	465	450	390	390	463	345	—	—	—	Wasted and weak but able to run, transferred on 16th day, recovered.
52	465	450	465	413	435	390	360	360	345	300	—	On 20th day very thin and weak but no paralysis; somewhat somnolent. Food changed. Recovered.

Although considerable loss of weight took place in each of these birds, none of the ordinary symptoms of avian beri-beri were noted up to the time when they became critically ill.

The condition seemed to be (after the first few days) one of simple starvation. It appears probable that, although at first some of the starch given was digested, as the increases of weight in some of the birds indicates, later on absorption ceased, possibly for mechanical reasons—the starch being apt to make a solid mass in the crop and duodenum. As will be seen later exclusive diets of glucose induced polyneuritis in birds and the addition of sago to a diet consisting of polished rice and yeast accelerated the development of the disease, Expt. XV (p. 345).

Experiment IX. Six fowls were fed on a daily ration of 30 *gms. starch plus* 1 *gm. of egg-yolk.*

TABLE VII.

No.	Day, and weight in grammes 1	9	11	13	15	17	19	Remarks
63	570	480	540	480	420	450	330	Died on 19th day. No paralysis.
65	570	540	540	480	420	—	—	Died on 15th day. No paralysis.
100	660	660	630	540	540	—	—	Died on 14th day. No paralysis.
94	1200	1050	1050	1020	1020	1020	900	Sick on 19th day, legs paralysed.
1	1370	1320	1290	1260	1200	1040	990	Died on 19th day. No paralysis.
10	1500	1340	1340	1380	1350	1350	1260	Quite well and strong on 19th day.

The egg-yolk in this experiment was mixed thoroughly into a paste with the starch and a little water, the mixture being given in pills. Although there were four deaths, in none of the four were characteristic signs of beri-beri observed previously.

One bird, however, developed beri-beri in the subacute form and one remained except for loss of weight quite well at the end of the experiment.

Cooper and Funk (1911) obtained somewhat similar results, pigeons fed on 1/10th to 1/40th daily rations of Coleman's starch, potato-starch, inulin, dextrin, and cane sugar in some cases developed typical symptoms of polyneuritis with considerable fatty degeneration in sciatic nerves and spinal cords. Many of the birds however only exhibited signs of weakness and then died, but even in these there was evidence of fatty degeneration. In all cases the deficiency in the diet of the anti-neuritic substance had evidently made itself felt, but in some cases at the time of death the depletion of the body store of active substance had only proceeded far enough to cause some degree of fatty degeneration, and was not sufficient to induce the development of the typical neuritic symptoms. The proportion of undoubted cases of polyneuritis is given below.

TABLE VIII.

Diet	No. of cases	Distinct cases of polyneuritis	Cases of weakness only
Coleman's starch	3	1	2
Potato starch (pure)	6	1	5
Inulin ,,	3	2	1
Cane sugar ,,	7	1	6
Dextrin ,,	8	5	3

The frequent failure of such carbohydrate diets to induce typical polyneuritis is surprising in view of the facts that the symptoms regularly develop in birds fed liberally on polished rice and even when polished rice or sago is added to a basal ration containing a minimum of the anti-neuritic substance (Experiments X to XV). In the case of starch, as already pointed out, the condition would appear to be one of simple starvation. The case of cane-sugar however offers more difficulty, as it would seem unlikely that saccharose escaped inversion in the alimentary canal. The excreta were however not examined for the presence of saccharose.

The results with dextrin show that exclusive diets of this carbohydrate frequently induced distinct symptoms of polyneuritis. This may be due to the fact that dextrin dissolves in water at ordinary temperatures and would be readily hydrolysed to glucose in the alimentary canal, so that the birds were thus called upon to metabolize large amounts of carbohydrate and depletion of their store of anti-neuritic substance rapidly took place.

Experiments with glucose have since been carried out, and it has been found that of four birds fed exclusively on aqueous solutions of this carbohydrate three developed typical symptoms of polyneuritis, while the fourth only exhibited symptoms of weakness with however considerable fatty degeneration in the sciatic nerves.

II. *The effect of increasing the carbohydrate ration upon the time elapsing before onset of polyneuritis in birds.*

The succeeding experiments show what happens when the intake of anti-neuritic substance is maintained constant, but the proportion of food-material is increased.

Experiment X.

(*a*) Six fowls weighing about 600 gms. each were fed on a ration of 30 gms. of padi daily (1/20th body-weight) for 45 days.

(*b*) Another six fowls were fed on the same ration of padi as the above plus 90 gms. of water-extracted unpolished rice (1/6th to 1/7th body-weight).

The birds of series (*a*) maintained their body-weight and were quite healthy at the end of 45 days, but those included in series (*b*) all developed polyneuritis in from 10 to 16 days.

Experiment XI.

(*a*) Ten fowls weighing about 600 gms. each were fed on 22½ gms. padi daily (about 1/25th body-weight).

(*b*) Ten other fowls received the same ration of padi and in addition 54 gms. of polished Siam rice daily (1/10th body-weight approximately).

Nine of the birds fed on padi only were strong and healthy at the end of 30 days, three of them gaining in weight and six losing in weight. The 10th bird escaped early in the experiment.

TABLE IX.

(*a*) Padi only, 22½ gms. daily.

No.	Day, and weight in grammes: 1	5	8	10	21	27	30	Effect of diet and % change by 30th day	
1	600	510	472½	450	372½	—	480	− 20 %	All healthy on 30th day.
2	600	630	570	510	630	—	705	+ 18 %	
3	600	480	450	360	420	—	375	− 37 %	
4	420	500	465	375	450	—	465	+ 10 %	
5	570	600	540	420	450	—	442½	− 23 %	
6	570	480	472½	390	420	—	442½	− 23 %	
7	540	450	420	360	435	—	412½	− 24 %	
8	495	600	540	537½	360	—	630	+ 27 %	
9	420	540	510	390	—	—	—	Escaped	
10	540	540	480	420	540	—	502½	− 7 %	

TABLE X.

Padi 22½ gms. + Polished rice 54 gms. daily.

No.	1	5	8	10	21	27	30	Effect of diet and % change in weight by end of exp.	
11	660	690	600	592½	600	—	615	Beri-beri 28th day. Died 29th day	− 7 %
12	540	570	510	510	540	480	—	Beri-beri 19th day. Died 29th day	− 11 %
13	390	480	420	390	375	—	—	Died suddenly at night 20–21st day	0
14	510	540	510	510	570	—	615	Beri-beri 28th day. Died 29th day	+ 20 %
15	570	570	510	495	555	—	555	Died acute beri-beri 30th day	0
16	630	480	480	435	472½	480	—	Beri-beri 25th day. Died 29th day	− 24 %
17	480	510	450	447½	420	—	420	Beri-beri 26th day. Died 29th day	− 12 %
18	600	570	540	525	600	—	735	Died acute beri-beri 30th day	+ 22 %
19	600	630	600	600	600	—	—	Died suddenly 20th day	0
20	570	540	480	450	480	—	—	Died acute beri-beri 30th day	− 16 %

The birds receiving polished rice as well all developed polyneuritis in from 20 to 30 days, five losing in weight, three remaining constant, and two gaining in weight. The results are set forth in detail in Tables IX and X.

[All these birds showed signs of early beri-beri—dullness, tremors, somnolence, and weakness on 18th to 19th day.]

The amount of anti-neuritic substance contained in 1/20th to 1/25th ration of padi was sufficient to prevent polyneuritis in fowls for at least 45 days, when fed exclusively on the diet (Experiment X), yet, although by the addition of washed polished rice to the diet the amount of active substance was actually slightly increased, the effect of the addition was to produce polyneuritis in less than 30 days. The conclusion clearly emerges that the amount of active substance required by the organism stands in some quantitative relation to the carbohydrate ration which has to be metabolized.

Experiment XII.

In this experiment birds were fed on small and large rations of water-extracted polished rice.

TABLE XI.

Series (*a*). 1/30th body-weight ration of rice for 1st 5 days, subsequently 1/12th body-weight.

	Effect of diet	% change in wt. by end of exp.
1.	Polyneuritis on 15th day	− 18 %
2.	,, ,, 15th ,,	− 20 %
3.	,, ,, 14th ,,	− 10 %
4.	,, ,, 20th ,,	− 40 %
5.	,, ,, 15th ,,	− 13 %
6.	Healthy for 24 days	0

Series (*b*). 1/5th body-weight ration of rice.

1.	Polyneuritis on 11th day	− 20 %
2.	,, ,, 16th ,,	− 16 %
3.	,, ,, 15th ,,	− 27 %
4.	,, ,, 14th ,,	− 7 %
5.	,, ,, 11th ,,	− 9 %
6.	,, ,, 15th ,,	− 3 %

The results show that, unlike what happened in Experiments X and XI, in which the anti-neuritic substance was provided in the diet, there was practically no difference in these two series as to time of onset of polyneuritis. It may be inferred that the lower scale of ration was already sufficient to produce the maximum effect in depletion of

TABLE XII. EXP. XIII.

Series I. Daily rations. Polished rice 1/25th initial body-weight. Yeast 1/2500th.

No. of pigeon	Effect of diet upon health of birds	Body-weights														% change in wt. by end of exp.	% change in wt. by 35th day, the average time taken for the birds receiving large ration to develop polyneuritis
		Initial	Day 8	16	21	32	42	49	56	63	70	78	84	91	95		
91	Strong and healthy after 95 days	350	340	330	320	310	290	270	280	280	250	250	250	300	270	−23 %	−11 %
92	Polyneuritis on 52nd day	440	430	430	410	380	—	350	—	—	—	—	—	—	—	−20 %	−13 %
93	Strong and healthy after 95 days	380	350	340	330	310	300	310	310	310	310	310	290	340	310	−13 %	−13 %
94	,, ,, ,,	460	390	390	370	340	340	310	340	330	310	300	300	340	320	−30 %	−26 %
			Day 7	14	21	28	35	42	46								
143	Strong and healthy after 47 days	310	340	230	300	270	290	290	260		...		...		...	−16 %	− 6 %
144	Slight polyneuritis on 39th day. No worse on 47th day	310	310	270	250	230	220	230	190		...		...		...	−39 %	−29 %

Series II. Daily rations. Polished rice 1/10th initial body-weight. Yeast same as above.

			Day 8	16	21	32	42										
97	Polyneuritis on 46th day	430	410	350	380	360	370		...		...		...		...	−14 %	−14 %
98	Polyneuritis on 33rd day	380	400	370	370	380	—		...		...		...		...	0	0
81	Polyneuritis on 22nd day	370	—	450	—	—	—		...		...		...		...	+22 %	+22 % on 16th
82	Polyneuritis on 42nd day	380	—	400	—	—	410		...		...		...		...	+ 8 %	+ 8 %
			Day 7	14	21	28	30	35	42								
149	Weakness in limbs on 39th day. Polyneuritis on 43rd day	330	350	340	320	320	—	310	300		...		...		...	− 9 %	− 6 %
150	Weakness in limbs on 39th day. Polyneuritis on 45th day	310	360	320	270	280	—	260	270		...		...		...	−13 %	−16 %
151	Polyneuritis on 27th day	360	420	370	340	350	—	370	—		...		...		...	− 3 %	+ 3 %
152	Polyneuritis on 31st day	300	—	280	250	240	270	—	—		...		...		...	−10 %	−10 %

the body store of active substance. Cooper (1913) obtained similar results. It was found that rations of polished rice varying from 1/30th to 1/10th of the body-weight induced polyneuritis in pigeons in about the same time (9 to 26 days), but when the rations fed were below 1/30th polyneuritis was delayed and did not develop until 21 to 33 days had elapsed. The maximum effect is evidently induced by a ration of carbohydrate of about 1/30th the body-weight of the bird. In quantitative investigations of the effect of various conditions upon the rate of onset of polyneuritis it is thus of importance to supply the organism with the minimum amount of vitamine and not to rely merely on the traces of active substance stored in the tissues. This point has frequently been overlooked in the past.

Experiments XIII to XV.

This series of experiments differs from those previously detailed in that all the birds were fed upon polished rice or sago with the addition of a constant amount of dried brewer's yeast sufficient to prevent polyneuritis for from 30 to 50 days when normal rations of carbohydrate were fed. By varying the carbohydrate ration and maintaining the supply of yeast constant, it was thus possible to study quantitatively the effect of increasing the carbohydrate component of the dietary upon the time elapsing before the onset of polyneuritis.

Experiments with pigeons.

The pigeons were first of all fed for three weeks on constant daily rations of whole barley equal to 1/20th their initial body-weight.

Experiment XIII.

Six pigeons were then fed daily on amounts of polished rice and yeast equal respectively to 1/25th and 1/2500th their initial body-weight. For birds weighing 350 gms. the daily quantities of rice and yeast were therefore 14 gms. and 0·14 gm. A second series of pigeons (eight) received daily the same proportion of yeast but two and a half times as much polished rice as the above birds, the rations of rice being thus 1/10th the initial body-weights.

Artificial feeding was employed in all the experiments.

Experiment XIV.

In this experiment the daily rations were as follows:

Series I. (Three birds.) Polished rice = 1/20th initial body-weight.
Yeast .. = 1/3500th ,, ,,
Series II. (Three birds.) Polished rice = 1/10th ,, ,,
Yeast .. = 1/3500th ,, ,,

For pigeons weighing 350 gms. the daily rations were therefore
Polished rice, 17½ and 35 gms. respectively.
Yeast .. 0·1 gm.

TABLE XIII. EXP. XIV.

Series I. Daily ration 1/20th initial body-weight polished rice and 1/3500th body-weight yeast.
Series II. ,, ,, 1/10th ,, ,, ,, ,, ,, ,, ,,

No. of pigeon	Effect of diet upon health of birds	Body-weights							% change in wt. by end of exp.	% change in wt. by 18th day, the average time taken by the birds on large ration to develop polyneuritis
		Initial	Day 5	16	26	37	41	53		
Series I.										
103	Polyneuritis on 37th day	390	390	380	350	300	—	—	−23 %	− 2 %
104	Weakness in limbs on 44th day. Polyneuritis on 53rd day	340	320	330	290	250	250	230	−32 %	− 3 %
			Day 7	14	21	28	37			
141	Polyneuritis on 32nd day	340	370	330	320	280	290	...	−15 %	− 3 %
Series II.										
145	Polyneuritis on 15th day	310	—	370	—	—	—	...	+19 %	+19 %
146	Polyneuritis on 16th day	300	370	350	—	—	—	...	+16 %	+16 %
148	Weakness in limbs on 22nd day. Polyneuritis on 26th day	320	400	350	320	—	—	...	0	+ 4 %

Experiment XV (with chickens).

The birds were first of all fed for three weeks on whole barley, the daily rations being equal to 1/20th their initial body-weight.

Five chickens were then fed on 1/45th their initial body-weight of polished rice and 1/2500th of dried pressed yeast, a chicken of 1450 gms. weight thus receiving daily 32 gms. of polished rice and 0·6 gm. of yeast.

Five other chickens received daily the same rations of rice and yeast as the above, but in addition 1/45th their initial body-weight of sago. Sago was used in this experiment instead of polished rice to facilitate

TABLE XIV. Exp. XV. *Chickens.*

Series I. 1/45th initial body-weight of polished rice + 1/2500 yeast.
Series II. ,, ,, ,, ,, ,, +1/45th initial body-weight of sago.

Effect of diet upon health of bird	Body-weights															% change in wt. by end of exp.	% change in wt. by 24th day, the average time taken by the birds on large ration to develop polyneuritis
	Initial	Day 5	12	19	22	34	41	48	55	62	66	83	89	96	100		
Series I.																	
A. Slight polyneuritis on 62nd day. Died.	1290	1180	1160	1120	—	1050	1000	970	950	880	—	—	—	—	—	− 32 %	− 16 %
B. Polyneuritis on 94th day	1450	1350	1340	1300	—	1250	1310	1250	1250	—	1270	1220	1230	1250	—	− 13 %	− 12 %
C. Polyneuritis on 97th day	1450	1350	1360	1310	—	1250	1240	1220	1200	—	1320	1160	1190	1190	—	− 18 %	− 11 %
D. Polyneuritis on 97th day	1430	1360	1370	1340	—	1260	1250	1220	1230	—	1280	1210	1280	1260	—	− 12 %	− 9 %
E. Healthy on 100th day	1300	1220	1240	1220	—	1180	1140	1100	1070	—	1100	1030	1060	1070	1070	− 18 %	− 8 %
Series II.																	
F. Polyneuritis on 31st day	1550	1630	—	—	—	1300 (31st)			...		...		...		...	− 16 %	0 to − 16 %
G. ,, ,,	1160	1140	1140	1160	—		...		.		...		...		...	0	0
H. Weakness on 13th day. Polyneuritis on 20th day	1570	—	—	—	1610		...		..		...		...		...	+ 2 %	+ 2 %
I. Polyneuritis on 22nd day	1300	—	1240	—	1200		...		...		...		...		...	− 8 %	− 8 %
K. Polyneuritis on 24th day	1770	1740	1770	1760	1850		...		...		...		...		...	+ 4 %	+ 4 %

artificial feeding, as sago passes the crops of birds much more readily than rice[1].

The results of Experiment XIII show that of six pigeons receiving daily 1/25th their initial body-weight of polished rice with 1/2500th of dried pressed yeast, three remained quite healthy for 95 days and even at the end of that time showed no signs of polyneuritis, one was healthy at the end of 47 days, and two developed polyneuritis in 39 and 52 days. The eight pigeons fed daily on 1/10th their body-weight of polished rice with the same amount of yeast as the above birds, however, all developed polyneuritis in from 22 to 46 days.

Experiment XIV shows that pigeons fed on daily rations of polished rice equal to 1/20th their initial body-weight with 1/3500th of yeast developed polyneuritis in from 32 to 44 days, but those fed on 1/10th their body-weight of rice with the same amount of yeast became ill in from 15 to 22 days.

Pigeons fed exclusively on the same polished rice in amounts equal to 1/10th to 1/30th their initial body-weight daily developed polyneuritis in from 9 to 26 days (Cooper, 1913). It is thus seen that, while the addition of a small amount of yeast to the polished rice diet could prevent polyneuritis in pigeons for a considerable time when the daily ration of rice did not exceed 1/20th the initial body-weight, when the ration of rice was increased to 1/10th, the yeast had little protective effect and the period elapsing before the onset of polyneuritis did not greatly exceed that elapsing when the diet was exclusively polished rice.

Experiment XV (with chickens) shows that of the five birds fed daily on 1/45th their initial body-weight of polished rice with 0·5 to 0·7 gm. of yeast one was still healthy on the 100th day of the experiment, three developed polyneuritis on the 94th to 97th days, and one on the 62nd day. The other five birds receiving in addition 1/45th their initial body-weight of sago daily fell ill with polyneuritis in from 13 to 31 days. As in the case of the previous experiments with pigeons, doubling the daily ration of carbohydrate thus greatly accelerated the development of polyneuritis.

The eight pigeons (Experiments XIII and XIV) fed on the small rations all lost in weight, while of the 11 pigeons fed on large rations

[1] It should be mentioned that during the first week of the experiment the rations of carbohydrate employed were somewhat larger, being 1/30th and 1/15th the initial body-weight of the birds. As some difficulty was met with in feeding these large amounts, the rations finally employed were as above.

(1/10th their body-weight) four gained in weight, three remained constant in weight, and the remaining four lost to as great an extent as some of the birds fed on the small rations and yet developed polyneuritis earlier. These results are of interest as they indicate that a dietary deficient in the anti-neuritic substance can sometimes maintain body-weight and even lead to increase in weight. Similarly, in Experiment XV of the five chickens fed on the large ration, three maintained their body-weight, but two lost in weight to as great an extent as the birds fed on the small ration and again developed polyneuritis considerably earlier.

The results thus confirm the earlier observations that the amount of anti-neuritic material required by the organism is not constant and independent of the quantity of food provided, but as the ration of carbohydrate is increased the demand of the organism for the active substance is also increased and if this be not adequately met, polyneuritis follows. When the carbohydrate ration was doubled (as in Experiment XV) by the addition of sago to a diet consisting of polished rice and yeast, the development of polyneuritis was accelerated considerably more than two-fold. Thus, when daily rations of polished rice and yeast equal respectively to 1/45th and 1/2500th the initial body-weight of the birds were fed, polyneuritis did not occur for nearly 100 days, but when the diet was supplemented by an equal quantity of sago, symptoms of polyneuritis appeared in from 13 to 31 days.

In this experiment the excreta of the fowls were collected daily, dried at ordinary temperatures by means of an electric fan, and the amount of undigested starch estimated by hydrolysis with acid and determination of the quantity of sugar produced by reduction (Bertrand) and polarimetric methods. The results, given in Table XV, were obtained.

The results show that in the case of the birds fed on the small rations the percentage of starch digested varied from 95 to 100 per cent., and in the case of the birds fed on the large rations from 90 to 100 per cent., the greater part of the starch fed thus being hydrolysed in the alimentary canal. Only small amounts of sugar could be detected in the excreta, and no dextrin was found at all.

Cooper (1914) found that alcoholic extracts of the excreta of a chicken fed on grain and the faeces of a rabbit fed on cabbage cured polyneuritis in pigeons, so that a possible explanation of the effect of large rations of starch in accelerating polyneuritis was that a portion of the starch was not digested, and interfered with the absorption of the

TABLE XV.

	Bird	Period during exp.	Starch fed	Starch excreted	Period during exp.	Starch fed	Starch excreted
Small rations	A	5th–11th day	204	5·0	12th–18th day	156	2·5
					19th–25th ,,	156	0
	B	,,	228	2·5	—	—	—
	C	,,	228	7·0	12th–18th ,,	162	9·0
					19th–29th ,,	250	6·0
	D	,,	228	1·0	12th–25th ,,	325	4·0
	E	,,	207	2·0	12th–33rd ,,	492	0
Large rations	F	,,	346	4·5	—	—	—
	G	,,	413	7·0	12th–33rd ,,	965	5·0
	H	,,	476	8·0	12th–25th ,,	674	0
	I	,,	325	3·5	12th–18th ,,	351	34·0
					19th–25th ,,	351	16·0
	K	,,	636	3·1	12th–18th ,,	468	36·0
					19th–29th ,,	720	8·0

anti-neuritic substance supplied in the diet. As however the amount of starch excreted by the fowls in Experiment XV was so small, it seemed improbable that the above explanation could hold at any rate for the particular rations employed in that experiment. Nevertheless it was decided to attempt to ascertain whether the anti-neuritic substance could be detected in the excreta. Pigeons were fed daily on 1/25th their body-weight of polished rice to which was added varying amounts of the pulverised dry excreta of chickens A, E (small rations of rice) and G, I (large rations).

Assuming only half the amount of active substance fed to the chickens was absorbed, the amount contained in the excreta administered should have prevented polyneuritis in the pigeons for 39 to 95 days (see Experiment XIII).

Experiment XVI.

TABLE XVI.

Pigeon	Amount of dry excreta fed daily	Effect on pigeon
1	3·5 g. of chicken A (small ration)	Weakness in wings and limbs 25th day. Polyneuritis acute 32nd day.
2	1·5 g. of chicken E (small ration)	Polyneuritis on 22nd day.
3	3·5 g. of chicken G (large ration)	Weakness in wings and limbs on 27th day.
4	4·5 g. of chicken I (large ration)	Weakness in wings and limbs 25th day. Acute polyneuritis 32nd day.
Pigeons fed on 1/25th body-weight of polished rice only		Polyneuritis in 20–28 days.

The excreta were evidently free from the anti-neuritic substance, and it thus seems that in Experiment XV the rapid onset of polyneuritis in the case of the birds receiving the large ration of carbohydrate was not due to imperfect absorption of the active material.

Experiments were next carried out with the object of ascertaining (1) whether the active substance is adsorbed by starch and (2) whether chickens digest the starch as readily when the ration is raised to 1/10th their body-weight (the maximum ration employed in the above digestion experiment being 1/20th the body-weight).

To 50 c.c. of a curative yeast extract 20 gms. of potato starch were added, and the mixture was allowed to stand at room temperature with frequent shaking for several days. The minimum curative dose of the solution for neuritic pigeons of about 300 gms. weight was determined before and after the addition of the starch, 10 animals being employed altogether. It was found that 2–3 c.c. of the solution before and after treatment with starch was just sufficient to bring about complete recovery, smaller doses having only a slight ameliorative effect. There was thus no evidence that starch adsorbed the active substance to any appreciable degree.

It seemed still possible, however, that with a much larger ration of starch the absorption of the active substances from the gut might be interfered with.

Four chickens were therefore fed on very large rations of polished rice (1/10th their body-weight) with the addition daily of 4, 6, $2\frac{1}{2}$, and $1\frac{1}{2}$ gms. of dried pressed brewer's yeast respectively. The excreta were collected daily, dried at 20° C. by means of an electric fan, and ground to a fine powder. The birds developed polyneuritis in the remarkably short period of 10 days. The amount of starch in the excreta was estimated and we were surprised to find that of this enormous ration the birds had digested 93 to 98 per cent., the amount of starch excreted per diem varying from 2 to 8 gms. Four pigeons were fed on rations of polished rice equal to 1/25th the body-weight and fixed quantities of the excreta. The amounts given daily were such that assuming provisionally that one half the yeast fed to the original chicken was excreted, they should have contained sufficient yeast (0·15 gm.) to prevent polyneuritis in the pigeons for from 39 to 95 days. (See Experiment XIII.) The pigeons however all developed polyneuritis in from 21 to 30 days, while control birds fed on the same ration of polished rice without the addition of excreta fell ill in from 20 to 28 days.

The addition of the excreta to the rice diet thus neither delayed nor

prevented polyneuritis in the pigeons, and this shows that the rapid onset of polyneuritis which results from the consumption of diets containing large rations of polished rice is not due to interference with the absorption of the anti-neuritic substance.

The main conclusion to be drawn from these experiments is therefore that the amount of anti-neuritic substance required by the organism increases with the quantity of carbohydrate *metabolized*. For the maintenance of health the intake of active substance must therefore be adjusted, as so to stand in some quantitative relation to the amount of carbohydrate included in the diet.

This fact is of practical importance in the prevention of beri-beri, as attention must evidently be paid not only to the absolute amount of anti-neuritic foodstuff incorporated into the dietary, but also to the proportion which this bears to the total carbohydrate ration. The exact ratio which must be maintained has not yet been determined, but the results so far obtained show that when the carbohydrate ration was doubled polyneuritis was induced more than twice as rapidly. Evidently then for the prevention of beri-beri the proportion of the anti-neuritic foodstuff in the diet must be maintained as high as possible, and large rations of foodstuffs deficient in the active substance carefully avoided.

Some minor points arising out of the results deserve mention. It was suggested previously (Cooper, 1914) that the curative action of extracts of excreta was due in some degree to the capacity of bacteria growing in the intestine to synthesize the anti-neuritic substance. Extracts of 2 gms. of *B. coli* (dry) however had no effect upon neuritic pigeons, but this could not be regarded as conclusive evidence, as so small a weight of bacilli was used. As, however, excreta are very rich in bacteria, the failure of as much as 1·0 to 5·0 gms. of excreta daily to prevent or even delay polyneuritis in pigeons fed on polished rice suggests that bacteria compared with yeast (0·15 gm. daily of which prevents polyneuritis for 39 to 95 days) contain an insignificant amount of anti-neuritic substance.

The results also show that birds can digest enormous rations of starch, amounting daily to 1/10th their body-weight.

SUMMARY.

(1) Chickens fed on 1/20th their body-weight daily of padi, cured or parboiled unpolished rice, or fresh (partly polished) rice, remain free from polyneuritis for at least 28 to 100 days.

(2) Chickens fed on the same ration of polished Siam rice develop symptoms of polyneuritis in from 20 to 70 days.

(3) When the polished rice is soaked in excess of water for 24 hours the birds succumb to polyneuritis in from 10 to 35 days; when the rice is soaked for 48 hours the disease appears still more readily, viz. in 15 days.

(4) Chickens fed on 1/20th their body-weight of parboiled rice which had been soaked for 24 hours also develop polyneuritis in from 13 to 39 days. This fact is of practical importance, as epidemics of beri-beri have been traced to the practice of soaking unpolished rice prior to cooking and discarding the water.

(5) Although birds fed on diets of polished rice, sago, or glucose develop polyneuritis, when fed on commercial starch they often fail to do so and merely lose considerably in weight. An explanation is offered.

(6) While fowls fed on 1/20th their body-weight of padi remain free from polyneuritis for at least 60 to 100 days, when this ration is supplemented by 1/6th the body-weight of washed unpolished rice or 1/10th of polished rice polyneuritis appears in from 20 to 30 days.

(7) On the other hand, chickens fed on rations varying from 1/5th to 1/30th of their body-weight of washed unpolished rice develop polyneuritis in about the same time, 10 to 20 days.

(8) On dietaries composed of rations of polished rice varying from 1/20th to 1/40th the body-weight and of yeast varying from 1/2500th to 1/3500th the body-weight pigeons and chickens do not develop polyneuritis until at least 32 to 100 days have elapsed, but when the carbohydrate ration is doubled by the addition of polished rice or sago the birds fall ill in from 13 to 46 days.

(9) Even when daily rations of polished rice as large as 1/10th the body-weight are fed to chickens together with varying amounts of yeast, 93 to 98 per cent. of the carbohydrate is digested and absorbed and the excreta contain no anti-neuritic substance. This shows that the rapid development of polyneuritis induced by feeding large rations of starch is not due to interference of undigested carbohydrate with the absorption of the active material.

(10) Intestinal bacteria, unlike yeast, contain little anti-neuritic substance.

Conclusion and practical application of the results.

The amount of anti-neuritic substance required by the organism increases with the quantity of carbohydrate metabolized. For the maintenance of health the intake of active substance must therefore

be adjusted, so as to stand in some quantitative relation to the ration of carbohydrate ingested, and it is when this necessary balance is not maintained in the dietary that beri-beri results. Although as ordinarily induced beri-beri can be described not inaccurately as a "deficiency disease," it may thus actually develop when a dietary containing an adequate amount of the anti-neuritic substance is regularly supplemented by a ration of a carbohydrate foodstuff deficient in this essential substance.

This is obviously of great practical importance in the prevention of beri-beri. Attention must be paid not only to the absolute amount of anti-neuritic foodstuff incorporated in the dietary, but to the proportion which this bears to the total carbohydrate ration. The precise relation which must subsist between the supply of active material and the amount of carbohydrate metabolized has not yet been ascertained, nor has it been determined for the other normal components of a dietary, *e.g.* protein and fat. It is therefore advisable to maintain the proportion of anti-neuritic foodstuff in the diet as high as possible, and large rations of foodstuffs deficient in the essential substance should be carefully avoided.

In the preparation of a dietary to obviate beri-beri, it thus becomes necessary to consider not merely its absolute content of anti-neuritic material, but also its total calorific value.

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