FURTHER EXPERIMENTAL STUDIES ON BERI-BERI. THE ACTION OF CERTAIN PURINE- AND PYRIMIDINE-DERIVATIVES.

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In one of my earlier papers on this subject(1), I was able to show, that a substance may be isolated from rice-polishings, which administered to birds, suffering from polyneuritis, produces a complete recovery.

The same substance has been isolated by analogous methods from yeast, milk and brain(2). The chemical properties of the substance suggested to me, that it probably belongs to the pyrimidine-group. As the substance is very unstable, an attempt was made to reduce the manipulations to the strict minimum and to avoid the action of alkali, which seems to have a very destructive effect. It was tried to simplify the method and to make the yield better, but all methods failed and I have to use again my former method. An extraction on a large scale is now in progress. I have also repeated the experiments of Suzuki, Shimamura and Odake(3) and I have found, that the method does not work in our conditions. With Malay polishings, stored very likely for a considerable time before their arrival in this country, I was completely unsuccessful.

It was thought, for instance, that the process could be shortened, by leaving out the precipitation with phospho-tungstic acid. A fractionation of rice-polishings, carried out on these lines, did not yield the beri-beri-vitamine[1], but a substance of similar properties, namely allantoin, which in the first case passes into the filtrate[2]. This substance was also tested on neuritic birds and was found not to be curative, but only to effect some improvement and to retard the death of the animals for several days. These results together with the probability of a pyri-

[1] This name being suggested for the anti-neuritic substance for the present (4), till the chemical structure is found out.

[2] Schaumann in a recent paper (*Arch. f. Schiffs- und Tropenhygiene* XVI. p. 825. 1912) claims to have isolated the anti-neuritic substance before me. The substance was not identified and the method used in his case could only lead to the isolation of allantoin, which, as I have shown, is present in rice-polishings in larger proportion, than the beri-beri-vitamine.

midine structure for the beri-beri-vitamine, led me to try the effect of known purine- and pyrimidine-bodies on neuritis in birds. These experiments had two purposes, first to see, whether these substances have a similar action to allantoin, the second whether the animal is able to use them for building up the necessary "vitamine." Besides this this investigation had to help the chemical investigation of the anti-neuritic substance, which has great difficulties because of the small yield.

The experiments which will be described in this paper show beyond any doubt, that a number of purine- and pyrimidine-substances possess a very marked action on neuritic birds. Although the sick birds rarely die later than 24 hours after the onset of the symptoms, as a number of controls have shown, I was able by addition of these substances to keep them alive for 4, 5 and often more days. The daily supply of 30 grm. rice with an addition of allantoin (synthetical product 0·025 grm. a day) delays the onset of symptoms for several days and lessens the waste of weight. The birds die with very slight or even without any neuritic symptoms. Similar to allantoin is the action of hydantoin especially as concerns influence on weight. Some substances have even a very marked effect on the symptoms, on several occasions results were obtained which could be regarded as real cures and lasted for 4–5 days. I propose to prepare some of these substances synthetically and to add them daily to the polished rice diet. Only a few of these substances used in these experiments were prepared by me. Most of them were supplied by Dr Levene, Professor Winterstein and Professor Schittenhelm, and I would like to express here my gratitude for their kindness. My explanation of the action of these substances is, that owing to a similar structure, they are able to replace some of the physiological functions of the beri-beri-vitamine.

They explain at the same time the action of nucleic acids on neuritic birds and also the action of certain alcoholic extracts (phosphatide fraction), which when free from the vitamine, contain as I have shown, a large amount of nucleic acid derivatives as impurity.

As uric acid has no effect on pigeons, it is made probable, that birds are not able to form allantoin from uric acid, a result which is in concordance with the fact, that their organs were found free from uricolytic ferment. In this respect the birds seem to show a great analogy to man. As Ackroyd(5) shewed, the amount of allantoin present in the food largely accounts for the amount of this substance secreted in the human urine.

The pigeons were fed as usual on polished rice; after the onset of symptoms a dose stated each time (as a rule 0·1 grm.), dissolved or in suspension in water was introduced in the crop and the animals closely observed. In other experiments (with allantoin and hydantoin) the substance was added daily and the animals weighed regularly, until they died.

Substance	Dose (grm.)	No. of animals	Origin of the preparation	Results
Uric acid ...	0·2	2	Kahlbaum	None.
Adenin ...	0·1	2	Winterstein	Survival 30–50 hours.
Guanin ...	0·1	2	Levene	Practically none.
Hypoxanthin ...	0·1	2	Winterstein	3–5 days survive.
Xanthin ...	0·1	2	,,	2–4 ,, ,,
Paraxanthin ...	0·1	4	,,	Cure and survival for 6 days, in other 3 cases no marked effect.
Uracil	0·1	2	Own prep.	Improvement of the symptoms and survival 3–4 days.
Thymin ...	0·05	2	Levene	None.
Allantoin ...	0·1	7	Own prep.	2–3 days } Very marked improvement in state of the animals.
Hydantoin ...	0·2	5	,,	2–9 days }
Yeastnucleic acid ...	1	1	Böhringer	4 days survival.
Thymusnucleic acid	0·2	2	Schittenhelm	Survival 9–14 days } Very marked improvement.
Guanosin ...	0·1	3	Levene	1–4 ,, }
Adenosin ...	0·05	1	,,	4 ,, }
Cytidin	0·05 of the nitrate	2	,,	2–6 ,, }

Duration experiments.

Allantoin experiment.

	30 grm. of rice+0·025 grm. allantoin daily			Controls, 30 grm. of rice		
Initial weight	I 379	II 322	III 357	IV 395	V 305	VI 319
4 days	406	315	372	361	298	312
8 ,,	363	276	338	323	258	288
12 ,,	317	263	322	294	244	240
16 ,,	303	256	308	276	226	—
18 ,,	↓	↓	↓	↓	190	
19 ,,	↓	↓	↓	232	—	—
20 ,,	296	246	292	—	—	—
24 ,,	296	247	—	—	—	—
25 ,,	272	242	—	—	—	—
% Loss of weight	28·3	24·8	18·2	41·2	37·7	24·7

From this experiment we see, that the birds with allantoin lose less in weight, show slighter symptoms. The birds with allantoin lose 1 grm. per 100 grm. weight, whereas those without 2·1 grm. daily.

Hydantoin experiment.

	Pigeons on 30 grm. of rice and 0·1 grm. hydantoin daily			Controls on 30 grm. daily		
Initial weight	I 367	II 371	III 406	IV 421	V 367	VI 385
After 2 days	365	391	413	416	360	384
4 ,,	357	404	420	437	368	399
6 ,,	351	384	424	403	352	395
8 ,,	343	376	404	391	336	376
10 ,,	327	364	398	378	313	356
12 ,,	330	350	389	365	305	355
13 ,,	312	↓	↓	↓	280	↓
14 ,,	—	340	375	348	—	340
16 ,,	—	336	368	340	—	327
18 ,,	—	337	352	341	—	313
19 ,,	—	305	↓	↓	—	↓
20 ,,	—	—	354	342	—	308
22 ,,	—	—	341	339	—	299
23 ,,	—	—	↓	300	—	↓
24 ,,	—	—	344	—	—	308
25 ,,	—	—	↓	—	—	266
26 ,,	—	—	339	—	—	—
28 ,,	—	—	—	—	—	—
30 ,,	—	—	291	—	—	—
% Loss of weight	14·9	17·7	28·4	29·0	21·5	30·9

Whereas the controls lost 1·33 grm. daily from 100 grm. weight, the birds with hydantoin lost only 0·98 grm.

CONCLUSIONS.

1. Certain purine- and pyrimidine-substances have a very marked effect on pigeons suffering from polyneuritis; a relationship between the chemical structure and the action could not however be established.

2. The action of allantoin suggests, that pigeons are not able to convert uric acid into allantoin.

REFERENCES.

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(4) Casimir Funk. Journ. of State Med. June, 1912.
(5) Ackroyd. Biochem. Journ. v. p. 400. 1911.