

Physiology. — *Experiments with JANSEN and DONATH's Antiberiberi-vitamin.* By Prof. Dr. C. EIJKMAN.

(Communicated at the meeting of March 26, 1927).

About thirty years ago I published in the Proceedings of the session of 29 May 1897 a communication from the Medical Laboratory of Weltevreden (Batavia) entitled „The Combating of Beriberi”. It included among other things a report about feeding experiments with gallinaceans, the results of which promised to throw a new light on the etiology of the disease to which so many Orientals succumbed, also in the Dutch Indies.

It had been demonstrated among others by WINKLER, that Beriberi brings on a degeneration of the whole peripheral nervous system, a polyneuritis or rather a polyneuratrophy. The symptoms of the disease tend to the same conclusion.

As to the etiology of the disease, however, researchers were still much in the dark. Truly, various theories had been advanced, but none of them gave satisfaction, for the prophylactic and the curative measures based on them yielded no or only little and temporary success.

At the time it appeared that a one-sided diet of polished rice could produce polyneuritis in gallinaceans, whereas unpolished or undermilled rice, the so-called silverlayer-rice, produced a sheltering as well as a remedial effect.

It has been the great merit of Dr. VORDERMAN, Medical Inspector in Java, that, after having got cognizance of the above-named results, he has instituted a special inquiry into the occurrence of beriberi in the native prisons, in connection with the feeding. It brought to light that in prisons, where polished rice was administered the number of cases of beriberi exceeded about 300 times the number revealed in prisons where unpolished rice was the staple article of diet.

VORDERMAN'S experience has since been corroborated by numerous observations, and even by advisedly undertaken experiments on man, also by researchers out of the Dutch Indies (British India, Japan).

The conclusion I felt confident to deduce from the animal-experiments, continued and extended by GRIJNS after my departure in the same laboratory, was that there must be a substance, or substances in the rice-polishings, responsible for the favourable effect. Indeed, from the polishings an active extract could be obtained that was amenable to further purification. After many vain trials by different researchers it was FUNK, who seemed at length to have succeeded in isolating the

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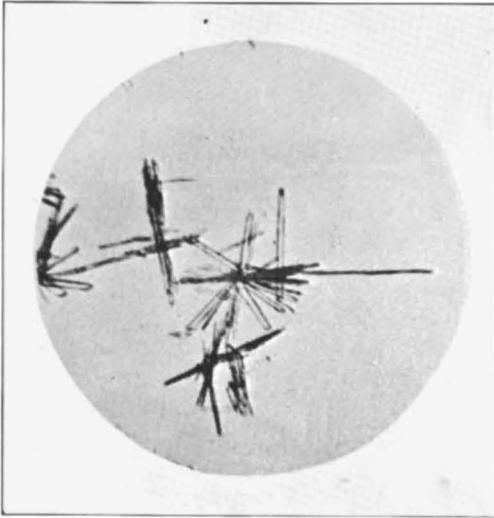


Fig. 1. Crystals of antiberiberi-vitaminchloride.

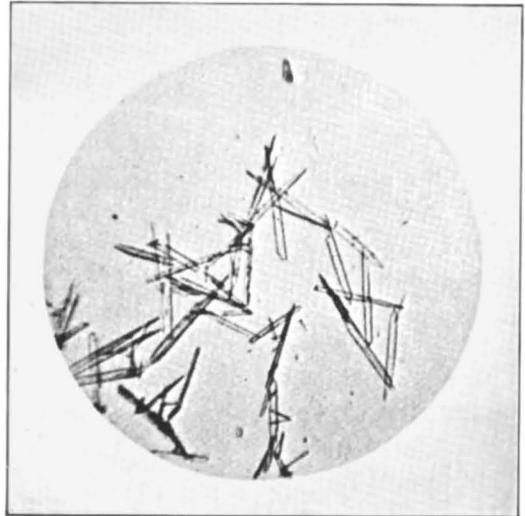


Fig. 2. Golddoublesalt.

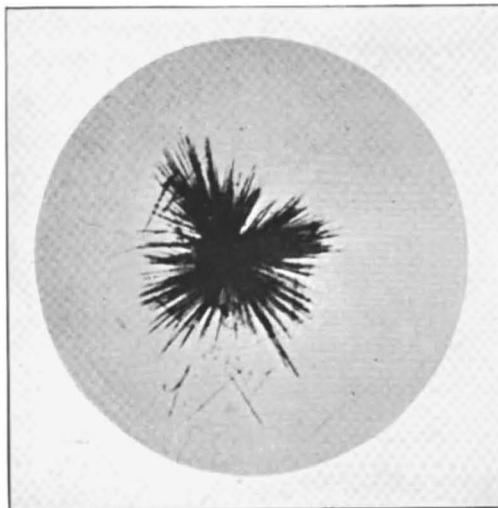


Fig. 3. Vitamin-picrolonate.

(From JANSEN and DONATH).

Enlargement 120 ×

active component in a pure, crystalline condition. He imagined it to be an organic nitrogen base of the formula $C_{17}H_{20}N_2O_7$. Its administration to an amount of 20 mgrs to pigeons, suffering from polyneuritis, had according to him a rapid curative effect. But afterwards neither he himself nor others have been able to confirm this. Moreover it has been proved that a favourable though only transitory effect can be produced by a number of substances in pigeons suffering from the disease, especially on timely interference, and specially when the substance is given not per os, but subcutaneously. However, these substances have no protective properties.

Meantime the name vitamin that FUNK has given to his substance, has become a generic name. A number of vitamins have now been distinguished, a deficiency of each of which in the diet is supposed to induce as many diseases, the so-called deficiency-diseases or avitaminoses.

Now, it may be called a happy coincidence, that in the same laboratory, where the first researches were made in this field, two of our countrymen JANSEN and his coworker DONATH have apparently succeeded, after many years of persistent endeavours, in isolating the antineuritis or, as they term it, the anti-beriberi vitamin from rice-polishings¹⁾.

They report that the addition of as little as 2 mgm., of the vitamin to 1 k.g. of polished rice, ergo in the ratio of 1 : 500.000, wards off polyneuritis gallinarum. I am in a position to confirm this in virtue of my own experiments with their preparation, to which I shall revert presently. At this rate 1 mgm of vitamin added to $\frac{1}{2}$ k.g. of polished rice would protect man from an outbreak of beriberi.

Some months back JANSEN and DONATH kindly sent me 40 mgms of their preparation, a white powder, consisting of microscopical needle-shaped crystals (Figs 1—3), well soluble in water, slightly less so in alcohol. The behaviour of the substance towards various reagents used in physiologic chemistry, in connection also with its elementary composition ($C_6H_{10}ON_2$), induces them to think it highly probably, that it contains either an imidazol-, or a pyrimidin-nucleus. The amount of the substance at my disposition being very limited I had to abstain from verifying these reactions. It seemed to me to be more interesting to supplement their experiments by applying the test of FOLIN and DENIS. This test, originally intended for a colorimetric determination of acidum uricum (blue stain with phosphotungstic acid and soda) gives, according to EDDY²⁾, also a positive result with extracts containing the antineuritic vitamin. It now appeared that a 1 0/0 solution of the anti-beriberi vitamin yielded with the reagent in question a blue stain, much weaker though than a uric-acid solution of the same concentration.

¹⁾ These Proceedings Vol. 29, No. 10, 1926. Erratum p. 1392 line 19 from bottom read 3 kg instead of 300 kg.

Geneesk. Tijdschr. v. Ned.-Indië, LXVI afl. 6, 1926.

²⁾ Proc. Soc. Exp. Biol. Med. 14, 164, 1917.

Now, in my feeding experiments I had to do without ricebirds, of which the researchers at Weltevreden could avail themselves. These birds yield the advantage that they react rather quickly on a deficiency of the vitamin in question with typical symptoms of polyneuritis, and that, owing to their light bodyweight, they require much less food, consequently also less vitamin than fowls and pigeons, which are generally used for similar experiments. On this account ricebirds were specially adapted to establish the vitamin-content of the various fractions obtained in the isolation-process. Besides, for examining the pure crystalline preparation JANSEN and DONATH have also taken pigeons. Their experiments were exclusively prophylactic, so that they had only to demonstrate that animals on polished rice, to which a certain minimum amount of their vitamin had been added, did not develop polyneuritis, even when they were submitted to a sufficiently prolonged experimentation.

I made curative as well as preventive experiments. According to my experience JANSEN and DONATH's objection, that a number of substances (which certainly do not contain any vitamin) appeared to have a slightly curative effect, applies only to a superficial improvement of short duration, at the most of a few days. The evidence is conclusive, however, when the experiment is continued for a longer space of time and complete recovery is the result. Contrary to them I, therefore, did not desist from curative experiments, especially with a view to their application to man. Fowls yielded hardly any favourable results with such substances. Also in other respects I consider fowls to be in casu first-rate experimental animals, although they require more food than pigeons, which is a drawback. With the latter the symptoms are rather variable. Most often the most conspicuous symptoms at the outset are convulsions and spasms of the neckmuscle, and not, as in cases of beriberi, paresis of the legs. Not rarely, however, do the pigeons succumb unexpectedly without the typical symptoms. As to the behaviour of ricebirds in this respect I must leave it undecided. In fowls the aspect of the disease is more uniform than in pigeons, it reminds us rather of that of beriberi in man, as KUENEN ¹⁾ also maintains. The progress of the disease is slower than in pigeons, so that there is less chance of the animal dying pending the experiment. Another objection raised by JANSEN and DONATH to the use of cocks, is that it often takes months before they manifest symptoms of polyneuritis. My experience is that this is not the case with half-grown cocks, as will be shown by the experiments described lower down, in which the incubation lasted with four cocks, fed on polished rice without vitamin, respectively 21, 23, 24 and 28 days. This, as we shall see, is even a shorter interval of time than the maximum with our pigeons.

Still, in pursuing the line of research of JANSEN and DONATH I first

¹⁾ Ned. T. v. Geneesk. 1917 I p. 2091 sqq.

of all experimented with pigeons. Whether the quantity of vitamin left after this, should be sufficient to experiment also with cocks, would appear afterwards.

Experiments with pigeons.

Nine pigeons were divided into three groups, in such a way that the total weight of the several groups inter se was about equal.

Group I serves as a test-group. Their trough is filled only with polished rice without vitamin. Bodyweight at the commencement of the experiment, resp. 285, 325, and 450 gms., with an empty crop, total weight 1060 gms.

Group II gets polished rice to which 2 mgms of vitamin-hydrochlorid per kg is added. Bodyweight respectively 325, 345 and 375, total weight 1045 grms.

Group III gets polished rice with 4 mgrs of vitamin-hydrochlorid per kg. Bodyweight resp. 295, 330 and 395 grms, total weight 1020 grms.

Every Wednesday morning the pigeons were weighed, after the food had been removed from the pen on the previous evening, as a full crop increases the bodyweight rather considerably. All are placed in separate pens.

On Dr. JANSEN's suggestion all the 9 pigeons were given, besides the rice ad libitum, every day (except Sundays) a pap consisting of $\frac{1}{2}$ grm of meat powder, profusely extracted with water, besides this a few drops of codliver oil some salt-solution and water. The meat for the supply of protein, the oil to prevent xerophthalmia.

JANSEN and DONATH have pointed out that the behaviour of the pigeons fed with rice + vitamin-hydrochlorid, is quite different from that of the pigeons fed with rice alone. The latter practically cease eating the polished rice after a few days, making a mess of their diet, while it looks as if they are rummaging for some grains to their taste, while the others finish their allowance with great relish.

This observation was substantiated by our experiments with pigeons. The pigeons of Group I which at first ingested about 20 gms per day, soon began to scatter the rice about and had to be fed forcibly, as they took hardly any food spontaneously. In spite of the forced feeding and a heightened meat-diet their bodyweight diminished steadily. In the second week one of the pigeons of this group has lost its usual energy and brightness, it sits huddled up in its pen, its feathers are ruffled. Already on the 16th day unmistakable signs of polyneuritis: spasms and convulsions of the muscles of the neck, paresis of the legs. The bodyweight has diminished from 285 to 215 gms. Now the animal is used for a curative experiment. To this end it is given 0.2 mg of vitamin-hydrochlorid per os. The apparent cures, effected by indifferent substances to which we alluded higher up, occur mostly with subcutaneous injection: So JANSEN and DONATH in following the example of THEILER found, that

even an injection of distilled water into diseased pigeons brought about a regression of the symptoms.

The small amount of vitamin was administered by the mouth at 12 o'clock in the day; at 2 o'clock the symptoms were still worse, but at 4.30 the cramps had ceased altogether. Now a trough with rice of group III (4 mg. of vitamin per kg.) is placed in the cage. Next morning the trough is empty, to all appearance the pigeon is restored to health. When this diet is continued, the appetite keeps good at first, the body-weight rises in the course of 17 days to that at the beginning of the experiment, then remains constant for about a fortnight, and then diminishes slowly, which is not surprising, considering such a prolonged monotonous diet. Then the rice of group II (2 mg. per kg.) is passed into the crop daily. After this curative experiment has been continued for 7 weeks in all, it is discontinued. The bodyweight has now fallen to 210 gm., without new symptoms of polyneuritis manifesting themselves. Subsequently the ordinary mixed pigeon-food; within a few weeks the bodyweight has risen above the initial weight.

It is a well-known fact that pigeons as well as fowls are very particular about alteration of food; they may enjoy their food ever so much at the beginning, in the long run they lose their appetite for a monotonous diet, though it may consist of the best foodstuffs.

At the beginning the other two pigeons of the test-group went the same way as the first, only more slowly, for although they sat huddled up, listless, and with ruffled feathers for more than a week already, no typical symptoms were yet visible on the 30th day of the experiment. Seeing that the bodyweight of either of them had fallen considerably in spite of the forced feeding (resp. from 450 to 300 grms and from 325 to 200 grms) and since we were afraid that they should succumb to inanition, a curative experiment was decided on in this stage. They were now given rice of group III (4 mgs per kg.). The result was an immediate recovery of the appetite, greater vitality, and a gradual increase of bodyweight. But after some time they fared worse, just as the first control-pigeon: less appetite, decrease of bodyweight, but polyneuritis did not make its appearance and finally improvement in every respect was brought on by ordinary foodstuffs.

The course of our experiment with the groups II and III differed widely from that with the control-pigeons. It is true, that in the long run their appetite also diminished, but the animals ate sufficiently to keep their bodyweight up to the mark in the 5 weeks that they were fed with vitamin-rice. For the rest they kept in good health. After this they got only polished rice without vitamin. The consequence was that the pigeons of group II revealed the typical symptoms of polyneuritis resp. after 20, 26, and 17 days. They were then submitted to the same curative experiment as the first control-pigeon, which again resulted in complete recovery.

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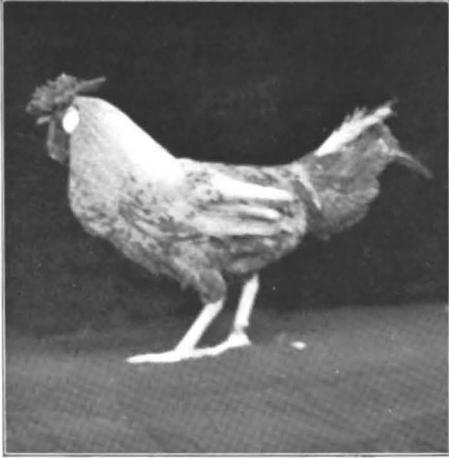


Fig. 4.

Cock having been fed for 4 weeks with polished rice + 1:500.000 vitaminchloride.



Fig. 5.

Cock with polyneuritis, after having been fed for 23 days with polished rice without vitamin. Gets then $\frac{1}{2}$ mg. vitamin per os.

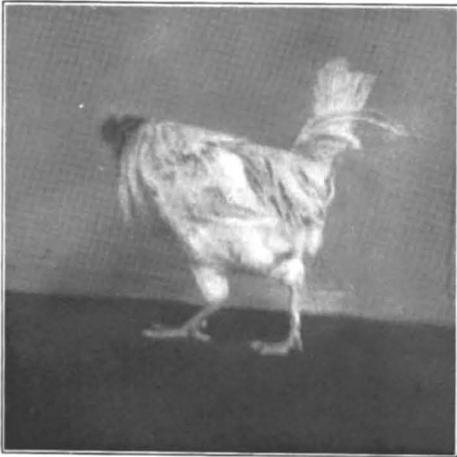


Fig. 6.

The same cock as in fig. 5, next day. Gets then polished rice + 1:500.000 vitaminchloride.



Fig. 7.

The same cock as in fig. 6, next day.

The pigeons of group III were more resistant than those of group II, very likely because they could lay up a larger store of vitamin in their organism. After they had been deprived of vitamin for 4 weeks, the experiment was discontinued. Indeed the pigeons were less thriving after that time, and their weight had diminished from 1050 in toto to 760 grms in spite of the forced feeding, but the typical signs of the disease have not appeared.

Experiments with cocks.

After the preceding experiments our store of vitamin had run so short that we had to economize.

Group I. Four young cocks serving for control. They get polished rice without vitamin. Bodyweight resp. 1400, 1575, 1735 and 1850.

Group II. Two young cocks. They are given polished rice with 2 mg. of vitamin-hydrochlorid per kg. Bodyweight resp. 1525 and 1540 grms.

In addition all the 6 cocks were given from the 16th experimental day 5 grms of meat-powder, 1 c.c. of codliver oil and some salt-mixture, in the way described in our experiments with pigeons.

Just as the pigeons in the preceding experiments the two groups of cocks show a marked difference in their behaviour with respect to the food, already in the second week. The control-birds eat their rice hardly any more of their own accord, they waste it and have to be forcibly fed. Nevertheless the bodyweight decreases, the crop discharges itself slowly, comb and gills hang loose and are pale. After an incubation of from 3 to 4 weeks, the four control cocks develop the typical paralytic form of polyneuritis. Their total bodyweight amounts to 5710 grms, against 6560 at the outset of the experiment. But for forced feeding the difference would undoubtedly have been considerably greater. The small remainder of our store of vitamin still sufficed to subject the cock that was the first to contract the disease, to the beginning of a curative experiment. On the 23^d day of the experiment, when the animal had been ill for 2 days, $\frac{1}{2}$ mgrm vitamin-hydrochlorid was administered per os (fig. 5). An appreciable improvement the next day; the bird is strong enough to stand again, but its gait is still unsteady (fig. 6); then rice of group II (2 mgrs of vitamin per kg) is passed into the crop and the next day the animal is slightly better again (fig. 7). In my wide experience with fowls the same recuperation never occurred spontaneously. I am convinced, that without the vitamin the condition would have deteriorated steadily, and the animal would probably have been dying or would have been dead already on the last named day. It was much to be regretted that, owing to the exhaustion of my supply of vitamin, its administration had to be put a stop to. The cock was then gradually restored to health by supplying yeast and the ordinary poultry food.

The two cocks of group II presented during the experiment quite a

different aspect from that of the control-animals, as had also been the case with the pigeons. They kept thriving, gained in weight although their appetite had diminished a little, forced feeding appeared to be unnecessary; comb and gills of a bright red and full of turgor. Such was their permanent condition until the 18th day of the experiment, the day on which the last cock of group I got ill. Thenceforth group II was supplied with rice alone, i. e. without vitamin. It was passed into the crop with 5 grms of meat-powder, 1 cc of codliver-oil etc. With this diet the cocks contracted polyneuritis after resp. 13 and 20 days. The predisposition of these animals to the disease, to which they had previously offered such a stout resistance, as compared with the control-animals, has thus been established. The comparatively brief incubation after deprivation of vitamin agrees in this respect with the behaviour of the pigeons of group II. Had our supply of vitamin been sufficient for feeding 4 mgrs per kg of rice to a third group of cocks, just as with the pigeons, the incubation period, after the deprivation of the vitamin would most likely have been longer also here.

All things considered we get an impression, that in the long run a supply of 2 mgrs per kg of polished rice would not suffice to keep up the store of vitamin proper to the healthy animal organism; 4 mgrs seems to be quite sufficient.

We find great satisfaction in stating that our experiments tend to corroborate the highly important results of JANSEN and DONATH's accurate researches. There is no doubt but that their isolated substance has the power to ward off polyneuritis gallinarum. We were able also to demonstrate its remedial power, as was to be expected. The authors have remarked with justice, that one cannot reasonably doubt of the purity of their substance, of which already a wonderfully small dosis yields the desired effect, and which could be isolated in the form of crystals, while the mother-lye proved much less active, I readily admit, that I had come to think (just as others had), that the protection from polyneuritis could not be ascribed to a single substance, but to a combination of two or more substances, notably because in our efforts to purify extracts, acting antineuristically, we ultimately came to a dead stop, where continued purification abolished the remedial action. This conception has been splendidly negated by the two chemico-physiologists of the Medical Laboratory of Weltevreden.

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