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Bacteriology. — "*Acetifying air- and ricebacteria the cause of Polyneuritis gallinarum*". By J. H. F. KOHLBRUGGE. (Communicated by Prof. C. H. H. SPRONCK).

(Communicated in the meeting of Januari 28, 1911).

The above title is at the same time a hypothesis which I intend to prove in the following pages.

It is a fact generally known and acknowledged that the discovery of Polyneuritis gallinarum by ELJKMAN, classified the so mysterious Beri-Beri in general with those phenomena that can be elucidated by experimental investigation. That Polyneuritis gallinarum belongs to the same group of diseases that includes human Beri-beri was likewise acknowledged by nearly all investigators; consequently experiments with poultry were made in order to learn how to fight the human disease.

By following the way taken by ELJKMAN, many investigators have brought to light a great number of facts, and yet the causa movens of the disease continued to be as mysterious as before, whosoever supposed that he had discovered it, might be sure that a subsequent investigator would declare it to be harmless.

In 1901 I was experimenting in Prof. ELJKMAN's laboratory about the autosterilization of the small intestine, and, as ELJKMAN was at the same time engaged in experiments regarding Beri-beri, this induced me, in order to study the intestine-flora of chickens, to examine, for economy's sake, the intestines of those chickens that had died of Polyneuritis gallinarum. As I examined however likewise some chickens that had been killed, I soon remarked that the intestine-flora and the intestine-sapreaction of these two groups of animals showed very apparent differences. Since that time I became convinced that the Beri-beriproblem should be studied from the intestines of poultry. Several investigators indeed have pointed out that presumably Beri-beri is caused by poisons formed, during digestion, by micro-organisms in the intestinal canal¹⁾. It was however astonishing that nobody consequently chose the intestines and their flora as point of issue, and that, if this was occasionally done (DUBRUEL, WRIGHT it had no result whatever.

On account of my return to Java I could not continue my investigations, but constantly, there and afterwards in Europe, I harboured the design of applying this method of investigation. Not

¹⁾ ELJKMAN, LAOH, MAURER, WRIGHT, HERZOG, JEANSELME, VAN GORKOM.

before Oct. 1910 I could accomplish this design, when Prof. SPRONCK was kind enough to place at my disposal a room provided with all that was indispensable for such an investigation, to be used as a laboratory. I set high value on the fact that I may add to this that Prof. SPRONCK is quite willing to declare that the nature and course of my investigations remained entirely unknown to him.

In order to examine chickens suffering from Polyneuritis gallinarum I had first to make healthy chickens ill, and succeeded in doing so, according to the method taught by EIJKMAN i. e. by compulsory feeding with white entirely prepared rice.¹⁾ After 18 or 19 days every chicken thus fed became ill, showing the wellknown symptoms of Polyneuritis gallinarum.²⁾

During these experiments I observed in the first place that during November and the first half of December the rice, neutral after sterilization,³⁾ grew sour within a few hours when it was exposed to the air. The first thing I wanted to know now was: Why does rice acetify?⁴⁾ Nobody could give an answer to this question, not even Prof. BEYERINCK of Delft, who kindly listened to my questions but felt even inclined to doubt the fact; I myself could however doubt no longer, for I saw that rice kept, after the sterilization, in well shut vessels remained neutral, whilst I saw it turn sour when it was exposed to the air in the different rooms of this new laboratory, where never before experiments concerning fermentation had been made. The rice turned also sour in the crop and in the intestines of chickens. From the above it appears that the rice did not turn sour by a simple chemical process. Consequently the rice must turn sour either by bacteria of the intestines or by bacteria of the air, or by both. By a series of experiments the obligate bacteria of the intestines of chickens were soon excluded, consequently had the acetification to be explained by the bacteria of the air. Now I made my investigations in that direction, but it was anything but easy to

¹⁾ In order to exclude however any idea of spoiled rice, the rice was sterilized at 120° before it was used. By so doing I obtained likewise a greater conformity with the often sterilized articles of food on board sailing-vessels that are so often accused of being operators of Beri-beri

²⁾ Frequent thin defecation, emaciation, paralysis, cyanose, dyspnoe (EIJKMAN).

³⁾ Uncooked not sterilized rice has always a feebly acid reaction.

⁴⁾ As sour fermentation was then for me only a sound to which I could not attach any idea, I applied in the first place to the vinegar-works "de Roos" at Amsterdam, where Mr. O. WIXFORTH in the kindest way furnished me with information and placed at my disposal the literature about vinegar fabrication; Mr. H. VLAANDEREN, a well known dealer in groats here, informed me about the different sorts of rice, the way of preserving rice etc. etc. To both my sincerest thanks.

isolate these micro-organisms that acetify rice among the mixture of moulds, fungi, and bacteria that fall out of the air on wet sterilized rice ¹⁾. This micro-organism however was found after all. It is a small, short rod, having great resemblance to the Colibacillus of the intestines. The isolation was rendered still more difficult because this little rod is very polymorph, and because a special medium and special breedingtemperature are required to obtain a good result.²⁾

This bacillus acetifies within 24 hours neutral sterilized rice, which but for this bacillus always remains neutral.

Just now I have compared it to Colibacillus, on the other hand it reminds me of the vinegar bacilli by its polymorphy. The more so, because it possesses the peculiarity, demonstrated by BELJERINCK in some vinegar bacteria, of showing on some special media easily deviating and hereditary qualities. So e. g. it is not difficult to deprive it of its power of exciting fermentation. According to BELJERINCK there are, also vinegar-bacteria that do no longer acetify, and must have originated in acetifying ones. Only on fit media, and when cultivated at the required temperature, and constantly having a new opportunity of acetifying rice, it is kept in good condition. It cannot be kept in rice, as it soon dies in the acid it has produced itself. Grow and multiply the bacillus does on the other hand on every medium that is not too sour, even though it afterwards dies or modifies itself in many of them. After domestication it accommodates itself to altered circumstances as to a feeding that was first refused or to a temperature at which in the beginning it was killed. Consequently we have here an exceedingly resisting bacillus, which could be cultivated in November and December in the whole laboratory. When however in December the temperature reached

¹⁾ At the present moment this is not difficult, since I found out that moulds and fungi only multiply on rice, when the rice has obtained an acid reaction by the bacterium causing rice to turn sour. Consequently the moment when the rice that is at first neutral, begins to show a feeble acid reaction, must be carefully observed, then, in most cases, pure cultures of the bacillus acetifying rice will be obtained by inoculation on specially prepared ferment. This is still easier in winter when the air of the room that has grown perfectly dry by the heat of a stove so that scarcely any moulds and fungi are found in it.

²⁾ The first inoculation from sour rice very often had not the desired result on the common alkalic or acid media at 37°C., very often they do grow, but lose their power of fermentation. Rice which is in the first place indicated as a medium cannot be made into a transparent medium. On ferment and malt the best results are obtained at a temperature of 17-23° C. On ferment results are obtained to a temperature as high as 40°, on malt not. I owe the prescription for malt to Prof. BELJERINCK, the recipe for ferments was used and accidentally tried in this laboratory. Only on rice, glucose and malt acid is formed.

occasionally and even repeatedly the freezing point, the bacillus disappeared almost from the air. It became so rare that cooked white rice could be exposed from 5 to 6 days to the air without turning sour¹⁾. Did it do so in the end, then the same short rods were always isolated from the rice.

On account of its polymorphy, and soon diminishing efficacy on artificial media, it was however desirable for me to dispose of a source from which every day new generations could be isolated. In December and January the air no longer produced them. As this bacillus seemed to have a special affinity for rice, the idea occurred to me that it might perhaps be found on dry grains of rice.

For this reason dry grains of rice were sown on sterilized neutral rice and really every grain of rice²⁾ proved to be a source of acid, in which the above mentioned bacillus was found. It seemed strange that this bacillus can live in such a perfectly dry grain. Therefore one might have surmised that it sticks perhaps only accidentally on it, as an air-bacillus. Further the finding of bacteria on or in all fully prepared grains of rice reminded too much of those investigators (VAN DIEREN etc.) who attribute all sorts of evil consequences especially Beri-beri to feeding with long kept, peeled grains. Both considerations induced me to examine unpeeled rice (gaba) in the following way. In order to exclude all air-bacteria every grain of rice (gaba) was separately passed several times through the gas-flame, then the coarse yellow skin, and the fine white one under it is charred, and only the interior part of the grain remains white. The grains are now ground in a sterilized mortar, and this mixture of carbon and white amylum is inoculated on neutral sterilized rice. Then it appears that the acetifying bacillus lives likewise inside the unpeeled grain of rice, and continues to live, when the rice is treated in the way we have just described, for the neutral rice grows sour, and the bacillus appears.³⁾

¹⁾ If this investigation had begun as late as December, I should never have observed the acetification of the rice, and consequently never have obtained the results I have come to now. Until now I have not yet been able to capture the bacillus from the open air, and it is by no means impossible that it is more correct to regard it as a wall-bacillus living inside the houses on the walls and spreading itself thence into the rooms.

²⁾ Common rice, white not glossy rice of Java, Moulmein, Rangoen and Bassein harbours this rice-bacillus, the latter more than the other, or a more vigorous variety than the other sorts.

³⁾ It is an interesting problem, when this bacillus enters into the rice, whether in the fields, or after it has been taken to the barn. This question must be solved in India or Italy. Perhaps rice cannot ripen without bacteria. What part do these bacteria act? These might be questions of as great agricultural interest as the

I followed the course of my own investigations by indicating only one bacillus, this is however less exact. Accidentally the before-mentioned short rod had first drawn my attention, and as every bacteriologist is anxious to work with pure cultures, it was everywhere isolated, in doing which I was often hindered by a certain lengthened rod that made its appearance in the cultures, for which reason several cultures were disapproved of. It appeared afterwards that in every portion of acetified rice and in every dry grain of rice both bacilli are found, which both make rice sour; it seems consequently that they live as in symbiosis, or support each other. Since that time I often worked with this mixture, indicated by nature, which appears to have a much greater vital strength ¹⁾.

If one keeps the rice of the noon-table in India till evening it may easily have turned sour, which proves that these bacilli acetifying rice occur likewise in the tropical regions; an investigation ought to be made whether their appearance is likewise subject to season or the state of the weather ²⁾.

I should like to communicate here what acid was formed by this fermentation. Dr. STAAL was kind enough to offer me to isolate and determine the acid; this investigation however is not yet finished.³⁾ I have likewise to thank Dr. STAAL for the information that in order to neutralize the acid produced by bacilli from 20 grams of dry grains of rice in 7 days 24.6 cub. cm. $\frac{n}{10}$ NaOH was required.

This investigation into the fermentation of rice was consequently finished, and relying on the reports of EYKMAN and others that rice turns sour in the crop of chickens, I passed over to the following working-hypothesis. The acetifying agens in the crop and intestines

well-known nitrogen-binding-bacteria for the roots of leguminous plants (BEIJERINCK). As Beri-beri does not occur in Suriname, I shall try to obtain rice from that colony for examination.

¹⁾ Now the question occurs to me, if not many of the unsuccessful results I obtained with media and breeding temperatures are to be attributed to my cultivating only with the short rod. Or are they after all two growing forms of the same bacillus? The vinegar-maker Mr. WIXFORTH told me that for his products he thinks pure cultures unfit.

²⁾ A rice-acetifying-bacillus was isolated by MAURER (Medan). I am convinced that his bacterium A, is closely connected to, if not identical with the one I isolated at Utrecht. MAURER's culture perished alas! I shall repeat the experiments described by MAURER in order to ascertain how far the conformity goes. ROST seems likewise to have found a similar bacillus in 1910.

³⁾ It is no *Acidum lacticum* (contra EYKMAN) and no *Acidum oxalicum* (contra MAURER). Nor is it volatile for it does not diminish by sterilization.

of chickens are not the common intestine bacteria, but the air-bacteria, described above, which acetify rice. A series of experiments followed from which appeared 1st. that the obligate intestine bacteria do not acetify rice, 2nd. that in the crop and intestines of chickens that died from Polyneuritis gallinarum the air-bacterium that acetifies rice can be shown; 3^d. that in acute cases of Polyneuritis the air-bacterium can almost supersede the intestine-bacteria.

Another working hypothesis followed. As, by feeding with rice Beri-beri presumptively results both in India and Europe¹⁾ from poisons developing themselves during the digestion out of food, containing amyllum (EYKMAN); as rice has a special affinity for the above-described acetifying air-bacilli, so much so, that they are even found in every dry grain: it may be admitted that these bacilli are the generators of Polyneuritis gallinarum not in the usual infectuous sense, but because they are pernicious to the body, when they turn the rice sour (in the intestines), either by the acid itself or by accessory products. If this supposition is correct, these bacilli must be harmless at subcutaneous injection, but they must cause Polyneuritis gallinarum when they are introduced into the intestines together with the food.

It was soon shown that these bacilli, and even entire cultures together, injected into the breast-muscles and into the peritoneum do not cause Polyneuritis gallinarum²⁾. The last and most important experiment remained:

I communicated already above that my chickens fed with sterilized rice, show after 18—19 days the well known symptoms of Polyneuritis gallinarum; ³⁾ EYKMAN who acted somewhat differently obtained the same result. ⁴⁾ If now the bacillus acetifying rice is the generator of the

¹⁾ A European Beri-beri epidemic was the one in the RICHMOND-asylum at Dublin. I do not know what food was taken there.

²⁾ Nobody will be astonished at the fact that chickens lose their appetite, if for days together 10-12 platina loops of these bacteria are injected into their peritoneum, even physiological salt-solution would make them ill. But they are not attacked by Polyneuritis, and do not die. Afterwards I injected into the breast-muscle which they could stand better.

³⁾ The first week they receive grains of rice which are strown into the chicken-house, soon they refuse this food, so that the 6th or 7th day one must proceed to compulsory feeding with sterilized rice-porridge.

⁴⁾ EYKMAN does not sterilize the rice, has the grains only ground and mixed with water, consequently the chickens receive the bacteria living in the grains of rice into their crops, when these are still alive. This is most likely the cause that EYKMAN often obtains stagnation of food in the crops by expansion and fermentation. I never had this result. Has the ground and moistened rice been kept a long time, it might occasion sudden death, as EYKMAN often observed.

disease, chickens fed with sterilized rice, and moreover with cultures of these bacteria, must much sooner be attacked by Polyneuritis gallinarum than the former, as these receive only such bacilli as accidentally pass from the air into the crops; ¹⁾ whereas the latter swallow with the rice entire cultures of these bacteria.

It appeared indeed that chickens, fed with sterilized rice and cultures of acetifying rice-bacilli bred on ferment, showed already on the 3rd day symptoms of paralysis and cyanosis. The third day they are sitting in the cage with paralyzed feet and bristling feathers, blue combs, show soon dyspnoe and die the fifth day. A dreadful diarrhoea was perceptible previously and the animals are enormously emaciated in those five days, so that even the breast-muscles have disappeared. All symptoms correspond entirely to those which chickens, fed with rice only, do not show before 24th or 25th day, but here they coincide in a short space ²⁾).

This experiment proved undubitably that the air- and rice-bacillus generating sour fermentation, isolated by me, can cause the symptoms of Polyneuritis gallinarum when it is introduced into the intestines of chickens.

The bacteria in question, the froth of the fermentation can be observed post mortem everywhere in the intestinal canal, the bacteria themselves seem sub finem to merge into the blood, and this fact explains that formerly so often bacteria were isolated from the blood of Beri-beri patients and chickens. Perhaps then already the same bacillus was found, which however was always rejected as the morbid agent, because it was supposed that it was to be expected that the morbid agent, when brought into the blood, must cause Beri-beri. It was not yet known that Beri-beri seems to belong to a peculiar group of diseases that find their origin in micro-organisms, and yet are no infectious-diseases in the usual sense of the word, and are best characterized as fermentation-diseases. In these diseases the morbid organism is only detrimental in the intestines and harmless in the blood. ³⁾). For the present this remains a theoretically construed group, among which I classify *Aphthae tropicae* ⁴⁾), the

¹⁾ Or the very bacteria of rice with EYKMAN's method. This is of course never the case with man, as he never eats raw rice. Yet I once saw a fanatical vegetarian do so, and I understand now why he perished from violent diarrhoea.

²⁾ By adding fewer bacteria to the rice the process can be rendered slower.

³⁾ This is the reason why DE HAAN and GRUJNS searched in vain in the serum of recovered Beri-beri patients, or in the serum and hydropericardial fluid of patients for "complementsbinding." Neither did they find any in chickens.

⁴⁾ According to Dr. MAURER's and my own investigations.

disease of BARLOW and scorbut.¹⁾ May they not remain long a theoretically construed group. Investigations with regard to scorbut have already been taken in hand. Now we should be anxious to know which fermentation-products generate the symptoms of the disease, this however is the task of the chemist rather than mine. At all events we have in the first place to wait for what STAAL's investigations concerning the acid that is formed, will teach us.

Another series of experiments related to the facts, discovered by my predecessors, that chickens fed either with unpeeled rice or with rice and raw meat, or with rice and Kadjang hidjoe etc. do not become ill at all, or do so later.

When cultivating the acetifying bacteria, it appeared that they develop themselves only in that part of the rice that by cooking separates a pultaceous matter, which we call "starch", and in India (adjen²⁾). This starch is analyzed by the bacteria into water, gas, acid and perhaps unknown products. The more starch is formed by cooking, the more luxuriously the bacteria grow in such rice, the less starch, the less food for the bacteria, the less formation of gas and acid.

Even by repeated cooking of rice the grains remain intact, the starch has however separated from them, and makes the grains stick together. and in these intervening spaces of starch one sees the bacteria grow rankly, and change it into water in which at last the grains float.

White fully prepared rice produces much starch, cooked gaba (unpeeled rice) produces hardly any starch, sterilized gaba again produces some more starch. The longer the unpeeled rice is cooked, the more starch is obtained, and consequently it is to be understood that (GRIJNS, MATSUSHITA) padi cannot entirely protect against Beri-beri. If one adds to fully prepared rice ferrihydroxid, eggs³⁾, spirits, animal charcoal⁴⁾, fresh meat, much less starch is formed⁵⁾. By

¹⁾ NOCUT and HOLST

²⁾ To the rice starch-works of J. DUYVIS I owe the communication that a not unimportant part of the rice cannot be turned to starch, a considerable residue remains which is sold as food for cattle, it contains 87,74 % of organic matter (STAAL). The percentage of starch of the various kinds of rice is very different.

³⁾ MATSUSHITA asserts that the addition of eggs to rice prevents Beri-beri.

⁴⁾ These least.

⁵⁾ Addition of these substances never impedes the growth of the bacteria and the fermentation, though it is inferior on account of the inferior quantity of starch.

anology I conclude that Kadjang hidjoe ¹⁾ will have a similar preventing influence on the formation of starch. ²⁾

Moreover it is by no means indifferent whether these substances are mixed with the rice before it has been cooked, or after the cooking has taken place. The starch that has been formed already ³⁾ cannot be precipitated, either the starch that is in process of formation is precipitated, or its development is prevented.

It is very remarkable that the natives, when left to themselves, never cook rice but steam it, whilst the steaming is variegated by washing, the consequence of which must be that the starch disappears, is washed away. Rice for prisoners and soldiers on the contrary is cooked, and though one tries afterwards by evaporation to give to the grains of rice the dry appearance that steamed rice has, yet the starch remains in the food. The first group is consequently guaranteed against Beri-beri, the other exposed to it. The danger augments considerably, if one eats by preference cooked or steamed rice, after it has entirely cooled down. On such rice the acetifying bacteria of the air have fallen down, not only does it smell sour, but it obtains that agreeable flavour reminding of fruit, a consequence of fermentation that is highly praised by gastronomers ⁴⁾. Food containing amyllum in which few substances are found that form starch guarantees against Beri-beri, and there exists moreover a sort of starch from amyllum that ferments, but through which only very little acid is formed. So our bacillus very easily produces water and gaz from starch of potato-flour, but by this process only a very small quantity of acid is formed. Consequently it is not strange that with potato-flour EIJKMAN cannot excite Beri-beri ⁵⁾.

A controversy arose between EIJKMAN on the one hand and GRIJNS with HOLST on the other, whether Beri-beri can occur when feeding with sterilized meal. I have not repeated these experiments but I draw the attention to the fact that our bacillus grows vigorously on

¹⁾ ROELFSEMA, GRIJNS, HULSHOFF-POL.

²⁾ The influence of Kadjang hidjoe and rice-bran may however be quite different, see below.

³⁾ For this reason the extract of dedek, or the salts found in dedek added to rice that has already been cooked, will not prevent Beri-beri (GRIJNS contra EIJKMAN).

⁴⁾ Tamil rice on the contrary which protects against Beri-beri stinks (FRASER, STANTON). It is submitted to a treatment by which the starch-substances are lixiviated, by this process the fermentation that gives an agreeable flavour is rendered impossible.

⁵⁾ Sterilized potato-flour ferments somewhat quicker.

⁶⁾ Nor is it strange that neither this flour nor gaba can absolutely protect against Beri-beri (GRIJNS).

sterilized meat, whilst it is quite superfluous to add anything to it ¹⁾).

Instead of feeding-experiments I took fermentation-experiments. Though I acknowledge that these need not cover each other entirely, yet until now they supplied nothing that is contradictory to the facts observed by feeding, so that my experiments perhaps may explain these facts and plead powerfully for the suggestion that Beri-beri rests entirely on fermentation processes ²⁾).

Moreover I think that it is of the greatest importance that these bacilli are found much more frequently in-doors than out-of-doors, that, in Europe at least, they are restricted to certain seasons, and that very dry air makes them disappear.

This fact explains why Beri-beri is more frequent near the sea-shore (VORDERMAN), with bad ventilation (VORDERMAN), in houses built in the European way (v. D. BURG); that expeditions in woody, marshy regions are notorious for the great number of Beri-beri patients (Djambi). Wherever the local conditions are favourable for the development, a so-called Beri-beri epidemy may break out, or a so-called Beri-beri house or ship may be found. If a house is strongly infected with these bacteria, the tenants can be attacked by Beri-beri from inhalation, from swallowing dryly, from drinking water, though they take their food (rice) outside the building ³⁾).

As the cultivation on various media and at various-temperatures taught me, how easily varieties may come into existence, and that the fermentation-process is moreover promoted by symbiosis, I suppose that varieties of this bacillus may exist that are extremely malicious. They obtain this quality most likely in the following manner. The bacillus must first accommodate itself to the medium,

¹⁾ A thick fleece of bacteria is formed at the surface of the fluid floating on the sterilized juice without acetifying it.

²⁾ It is by no means my intention to explain all symptoms, observed by monotonous feeding, by the fermentation excited through the bacillus. The want of nucleoproteides or nucleine (JEBBINK, NOCIT, SCHAUUMANN) may either predispose or promote the disease or be in itself the cause of it. This nucleine theory is not attacked by my investigations. A bridge might be built across, if it could be proved that nucleoproteides are analyzed by fermentation. In that case fermentation would not produce active destructive substances, but death would be caused by depriving the body of the required food. This theory appears to me very admissible, as up till now, I could not succeed in making chickens sick by inter-muscular injection of the pure fermentation fluid.

³⁾ So in the building of the doctor-djawaschool at Batavia (1900) 30% of the pupils who took their meals outside the school became ill. Would perhaps new walls offer a fit medium and this perhaps be the cause that rice turned so quickly sour in this new laboratory? BENTLEY was likewise of opinion, that the generator of Beri-beri must be a wall-bacillus.

and the temperature of our intestines, when it has succeeded in this, and leaves our body with the faeces (VAN GORKOM), it will operate much more strongly when it enters into another man's body (WRIGHT). In this way, I think, I can likewise explain why the disease seldom develops itself suddenly, and yet in other cases can assume such an acute form (Djambi). In such extra-ordinary cases it mocks all prophylactic measures, though they may be ever so rational (HULSHOFF POL)¹⁾.

So far about the aetiology of Beri-beri. A single word more about the therapy. The latter can be divided into prophylactic and causal therapy.

We know already very much about prophylactic therapy. We know that Tamil-rice and red rice usually protect against Beri-beri; we know that a nourishment more in accordance with the European usages suppressed Beri-beri in the navies of the Dutch Indies (VAN LEENT) and of Japan; JEBBINK has likewise shown in his dissertation the great difference there is between the two methods of nourishment. Here I shall only emphasize the fact that the native soldier, who is much more subject to Beri-beri than the European soldier, receives in his food more substances containing amyllum than the latter. Moreover we know already, and the investigation lying before us confirms it that for fighting fermentation, the way in which rice is prepared is of great importance. One should always try to apply a method by which the starch is washed away. Further one should examine, if the starchy substances can be removed before the rice is transported to the barn and peeled. Most likely the preparation has much greater influence than the age after complete decortication or the bringing into the barn of fully prepared rice (VAN DIJREN contra EYKMAN and GRIJNS).

Other experiments have taught that the addition of certain substances to the cooked rice prevent the appearance of the disease. LAOII mentions the side-dishes usually taken by the natives, others praise especially kadjang hidjoe (HULSHOFF POL), EYKMAN rice-bran, SCHAUAMANN ferment. When cooked directly with rice these substances

¹⁾ Consequently I do not think strange at all that WRIGHT could make monkeys suffer from Beri-beri by feeding them with rice and banana, which had been rubbed on the floor of sick-rooms where Beri-beri patients were nursed, whilst subcutaneous injections remained unsuccessful (HUNTER, KOCH). Transportation of the disease by ships and men to regions that had hitherto been exempt from Beri-beri can be explained, if it may be admitted that rice in those regions did not show the same fermentation phenomena, or that a specially virulent variety had been introduced which had become extremely active on account of its passage through the human body.

might, as mentioned before, prevent the formation of starch, but if I rightly understand the investigators, they did not act in that way : consequently this explanation does not hold good. Moreover they might hinder the growth of the bacilli or prevent fermentation. I know by experiment, that these substances when alcalised do not hinder the bacilli, they prevent the growth however undoubtedly by the acid they contain themselves.

If on the contrary we admit that the symptoms of the disease depend on the fact that the fermentation in the intestines does not produce active poisons but deprives the body from feeding-substances (НОЧИТ. SCHAUWMANN) without which it cannot continue to exist, it would be possible, that these were added again to the body by the beans called kadjang hidjoe etc. This point requires further investigation.

Causal therapy will try to fight the bacilli¹⁾ and the fermentation and its products in the intestinal canal. In this direction however as yet no experiments have been made with the exception of those with the above mentioned articles of food. Causal therapy can take another road by counteracting the influence of substances that have eventually crept into the blood, or by supplying such substances as may have been withdrawn from it. I know that Prof. EYKMAN is making experiments in that direction.

In view of the observations of my predecessors, of my own investigations and of the fact that, in so far as I have hitherto been able to apprehend, neither the injection of the bacteria, nor that of the filtrate of acetified rice (interperitoneal or inter-muscular) excites Polyneuritis in chickens, I must admit that the bacilli causing fermentation deprive, from the intestine canal, the body of substances, by which the quick emaciation must be explained. (500 gr. in 4 days) at a slower process the symptoms of paralysis show themselves first, but at all events the withdrawal of these substances ultimately renders life impossible, and death is the consequence²⁾.

If really, as now-a-days is generally admitted, human Beri-beri and Polyneuritis gallinarum is to be attributed to the same or akin causes,

¹⁾ MAURER thinks he can obtain this by acids (acidum lacticum, muriaticum, phosphoricum). As the bacillus soon dies in acidiferous media, the acid produced by the bacillus itself applied in great abundance might act as a curative.

²⁾ In all therapeutical experiments it is strictly required to make with HULSHOFF POLL a sharp distinction between the symptoms proper of Beri-beri and the subsequent consequences, on account of the degenerated nerves. Only against the former we may expect to find an active remedy. Moreover not all that we remark in chickens is applicable to mammals, as HOLST's experiments with rice feeding of Cavyas have shown.

³⁾ YAMAGIWA is of opinion that the regressive metamorphosis is a consequence of anaemia.

it must be possible, in case of acute Beri-beri, to isolate such like bacteria from the faeces of the patients. It is however possible that they perish in the rectum, as is the case with Polyneuritis of chickens, in whose whole intestine canal very often the acetifying bacilli are exclusively found, and yet it is very difficult to detect them in the Coecum. Consequently we shall have to wait for a favourable case, in which post mortem a fresh stomach and the small intestines can be examined ¹⁾. Should even under the most favourable conditions (acute death in the first stadium) the bacilli not be found, either human Beri-beri and Polyneuritis gallinarum is not the same, or there are different generators of the many diseases that have been classified with the group Beri-beri. Yet I hope that, even in this case, the results lying before us may be of use to ascertain the aetiology of these diseases, which are, at all events, closely allied to Polyneuritis gallinarum. Though a preliminary communication does not require that the literature of the subject is reproduced in it, yet I have taken account of the literature in order to avoid prejudicing prior rights of others, and to control my own results. Prof. EYKMAN was kind enough to place his collection of separata at my disposal, for which kindness I offer him my sincerest thanks. Much literature is likewise found in the book of DÜRCK.

I foresee that, when my results are controlled, the fact that it is exceedingly difficult to cultivate the bacillus pointed out by me, with conservation of its virulence, will excite the most important criticism. Moreover the bacilli (from air or wall) isolated from sour rice act much stronger than those isolated from rice-grains. Other differences depend on the seasons. Experiments with bacilli that have already been modified will of course give other results. I hope however to succeed in finding a method enabling us always to dispose of vigorous bacilli. The capriciousness or variability of the bacillus reminds us of the fact that likewise the evidence of the symptoms of Beri-beri with rice-feeding is exceedingly capricious, with regard to the earlier or later date of its appearance, as the protocols given by EYKMAN and HOLST show (differences of three weeks). This resistance is attributed to the animals, which may be true but has not been proved.

I give here these preliminary results, as my personal means do not allow me to continue the required experiments with different articles of food, and the different methods of preparing rice in every detail. Perhaps others disposing of ampler means will be inclined to repeat these experiments, and to bring them both here and in India to the end wished for.

Utrecht, 27 January 1911.

¹⁾ According to VAN GORKOM, WRIGHT, DUBRUEL human Beri-beri always begins with inflammation of the mucous membrane of the stomach and the intestines.