UNIVERSITY OF GLASGOW,

THESIS FOR DEGREE DOCTOR OF MEDICINE.

A CONTRIBUTION TO THE ETIOLOGY OF BERI-BERI.

BY

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My very best thanks are due to Dr. G.S. Middleton who so kindly placed at my disposal his two cases of Beri-Beri, as well as their clinical histories contained in his ward journal.

I am also much indebted to Dr. Steven, to Dr. Workman, and to Dr. Barlow, for the use of their laboratories and apparatus.

With this thesis I submit a series of Microscopic specimens which will serve as illustrations to some of the points referred to throughout its pages.

I.

That the symptoms and signs of beri-beri are due to a Multiple Peripheral Neuritis, is I think now an accepted fact. And the question which remains at issue, if indeed it has not yet been settled, is - What is the cause of this Neuritis?.

A Multiple Neuritis is symmetrical and being so the Parenchymatous degeneration of the affected nerves must be due to some irritant circulating in the blood, this irritant having a special affinity for the nerve protoplasm. And the peripheral parts of the nerves are the parts most affected because these being farthest from their source of nourishment, their vitality will be proportionately less, and they will therefore the more readily undergo degeneration.[1]

We are familiar with many examples of Multiple Neuritis caused by poisons circulating in the blood. Alcoholic Neuritis, Arsenical neuritis, the neuritis of small pox, of enteric fever, and certain aspects of diphtheritic paralysis, may all be cited by way of illustration. In the first two cases, alcoholic and arsenical/

arsenical neuritis, the poisons get into the circulation from without by means of the digestive system. In the other cases, or at least I may say without fear of contradiction, in the cases of diphtheria and enteric fever, the toxins are manufactured in the blood or tissues, the result of the vital activity of certain specific micro-organisms.

Early in the study of beri-beri it was recognised that its symptoms must be due to some deleterious if not actually poisonous substance present in the circulation. With this in view many and various causes were mentioned by way of explaining the etiology of the disease. It was thought to be allied to rheumatism, to scurvy, to be a form of Malaria. Wernich examined the blood in many of his patients and concluded that the disease was similar to pernicious anaemia. This theory he maintained, would explain the cardiac failure, the dropsy, and the nervous and other symptoms. But it was found that the changes he described in the blood were by no means common to all cases of beri-beri, and that indeed many of these changes did not in reality exist, but only in his preparations, the result of his faulty methods of examination.

Diet/

Diet again, was claimed to be the cause. It was said that many of the sufferers lived entirely on rice, and that on substituting a more nitrogenous food, they showed marked signs of improvement. Others maintained that the diet was too rich in nitrogenous material and claimed an improvement on giving less. Impure water, overcrowding, gases from organic decomposition, were all given an important place among the causal factors of the disease.

But a more adequate explanation than any of these had to be found, for not one of them was convincing or satisfactory. In some of the other infective diseases a micro-organism had been found in the blood & tissues of those affected. These organisms in growing produced certain poisons called toxins, and it had been shown that the poisons so produced, were the causes of the characteristic symptoms of the disease. The task therefore which presented itself was to find in the blood or tissues of people suffering from beri-beri, a specific micro-organism with characters analogous to the organisms of such diseases as diphtheria or enteric fever. Some idea of this kind was evidently in Simmons's mind twelve years ago, when he wrote as follows:-

follows:- "Ten years study and observation of the malady (beri-beri) under a great variety of circumstances and conditions, have led me to the definite conclusion that its exciting cause is a specific poison or germ, having many striking resemblances in its mode of production to paludal or Marsh miasm, though entirely distinct and separate from it."

Various investigators have discovered micro-organisms in the blood and tissues of patients suffering from beri-beri, but unfortunately the variety of these organisms is very considerable. Pereira[3] in 1874, reported the presence in the blood of micro-organisms which he described as micrococci in rapid motion. He says he also found these in normal blood, and so he considered them as of no importance. It is therefore probably De Lacerda[4] who deserves the credit of being the first to describe an organism which he considered to be the cause of beri-beri. This was a bacillus, the same, he says, as he cultivated from rice, and the same as he found in the blood of rats which had died after eating the rice. Some years later however, (in 1887), De Lacerda[5] cultivated cocci from the blood of his beri-beri patients, and produced by inoculating rabbits/

rabbits the symptoms of that disease.

In 1885 Ogata[6] found in the spinal cord of one who had died of beri-beri, a bacillus something like the Anthrax bacillus. Growing in gelatine it had a "tree-like appearance" and on agar-agar a "yellow grey-white colour." On injecting cultures of this bacillus into mice and dogs he says there ~~was~~ were developed in them the symptoms of beri-beri. He cultivated the organism on a medium of gelatine and grape sugar, and found that the fluid resulting from this growth on being injected into animals caused paralysis. He found his bacillus in the intestines of beri-beris and concluded that it was the toxins of these bacilli, which being absorbed into the circulation, caused the paralytic symptoms.

In 1887 Van Eecke[7] reported that he had cultivated four different kinds of organisms from the blood of his patients. These were as follows:-

(1) "Micrococcus albus", a micrococcus which was immobile, aerobic and liquified gelatine.

(2) "Micrococcus tetragonus flavus", cocci arranged in tetrad form.

(3) "Micrococcus flavus", also aerobic and liquifying gelatine.

(4) "Bacillus/

(4) "Bacillus flavus", short rods in pairs and chains, in active motion, aerobic and with spore formation.

On injecting animals with these growths three of them, the first, third and fourth, each produced paralysis. No result was obtained with "Micrococcus tetragonus flavus."

However important all the above observations might be, it was not till Pekelharing and Winkler[8] took the subject in hand that anything like a satisfactory account of the Bacteriology of beri-beri was given. In the first place, at post-mortem examinations, an organism was sought for in the tissues and cerebro-spinal fluid, but with no conclusive result. On investigating however the blood of their patients while alive, the result was much more satisfactory. For in the blood were found numerous micro-organisms, both cocci and rods, and these were demonstrated in fresh blood, as well as in stained specimens. An extended inquiry as to the condition of the blood in people residing at Atjeh and at Batavia, was next undertaken. In the former place where beri-beri is very prevalent, most of the inhabitants had "cocci and Bacteria" in their/

their blood whether they were actually suffering from the disease or not. At Batavia on the other hand, where beri-beri was almost never met with, the blood of its residents showed total absence of these organisms. As regards the micro-organisms in the blood of the so-called healthy people in Atjeh, it was pointed out that almost no one enjoyed good health there. Most of its inhabitants complained of a feeling of heaviness lassitude, palpitation, pains, and often loss of tactile sensation in the legs. The doctors who were carrying on the investigation, also experienced these symptoms and they were not free from them till they removed to Batavia. Fresh arrivals at Atjeh had no micro-organisms in the circulation but these could almost always be demonstrated after a short residence, disappearing however on removal from the infected neighbourhood. Thus all these observations gave confirmation to the surmise that beri-beri was due to the micro-organisms present in the blood.

The next step was to cultivate the organism or organisms outside of the body. This was found to be a matter of considerable difficulty. Cultures were taken from the blood of 80 patients, in all of whom the organisms were known to be present. Of the 80 tubes only 15 of them showed any growth and not all of these growths were alike. Of the 15, twelve were

were micrococci and 3 were rods. Of the 12 micrococci 9 seemed alike and they showed a close resemblance in many of their characters to Staphylococcus Pyogenes Albus. Two more were yellow growths; the twelfth was not well made out. The growths in the three tubes with rod-shaped organisms, differed considerably from each other. It is to be noted that of the 9 white micrococci, 6 liquified Gelatine and two did not; the ninth was lost. In addition to cultivating these micrococci from the blood Pekelharing and Winkler had no difficulty in growing a similar organism from the air of houses in Atjeh.

It was next necessary to test the pathogenic characters of these micro-organisms. Single injections of cultures produced no effects on the animals. But this was quite to be expected for the disease beri-beri differs from other infective diseases in that while most of the others require but one exposure to their infecting agent, it is necessary to have a prolonged contact with the virus of beri-beri, to insure the development of that disease. And so the injections were given at intervals, and were continued over periods extending from 9 to 48 days. Seven rabbits and two dogs/

dogs were treated in this way with cultures of the white micrococcus. Six out of the seven rabbits and one of the two dogs showed on post-mortem examination well marked degeneration of their nerves. From the blood of these animals the same micrococcus was cultivated, and this in its turn produced a nerve degeneration in other rabbits. Cultures of the white micrococcus were given to rabbits among their food, and diffused in the air they breathed, with results similar to those obtained on injecting this virus. Again, saline solutions impregnated with the air of a room in Atjeh, were injected into a rabbit. This animal died and the post-mortem showed a degeneration of its nerves. Micrococci too werecultivated from this rabbit's blood and these produced degeneration of nerves in other animals. Control observations were made, the control animals being kept under circumstance exactly similar to those receiving the injections. No nerve degeneration was found in the former. A rabbit was injected with a culture of Staphylococcus pyogenes albus. The rabbit died in five days. Abscesses were found in its liver and kidneys but no degeneration in its nerves.

The/

The pathogenic properties of the three rod forms and the two yellow cocci cultures obtained from the blood, were in a similar way investigated, but there was no certain evidence to show that these caused a nerve degeneration.

The conclusion therefore that Pekelharing and Winkler arrived at was that the "white micrococcus" they cultivated from the blood, is the cause of beri-beri.

As to its liquifying gelatine or not, that they thought to be a matter of no moment, but to depend mostly on the culture medium on which the organism was grown. The rod forms were supposed to be extraneous probably impurities. The "yellow cocci" they seem to think in some way related to the "white cocci", but on this point they are not very clear.

They believe that the "white cocci" impregnate the air of the infected houses, ships and districts. These cocci get into the human circulation through the air passages and when absorbed in sufficient amount— some week's exposure to the infection being necessary— they produce in the blood, toxins in such quantity and of such a nature, as to bring about a parenchymatous degeneration/

degeneration of the peripheral nerves.

Following on these observations of Pekelharing and Winkler, came Eykmann[9] (1888), but he could not confirm their conclusions. He himself got only negative results from the study of the Bacteriology of beri-beri. Neither could Mendes[10] agree as to the specific nature of the cocci of Pekelharing and Winkler. In 1887 Sternberg[11] when in Rio de Janeiro, tried to cultivate an organism from the blood of four typical cases of beri-beri, but his results were also quite negative. Leopold[12] in 1892, isolated four micro-organisms from the blood of his patient. These were as follows:-

(1) "Staphylococcus pyogenes albus."

(2) "Micrococcus of chain form".

(3) " A small Streptococcus, colourless, of unknown character and difficult cultivation".

(4) " Micrococcus, with by inoculation in animals—guinea pigs and dogs—causes a degenerative Neuritis",", and is described as the typical micrococcus of beri-beri. But the account by Leopold of his observations is otherwise so indefinite and unsatisfactory that one cannot give much heed to it.

In 1893 Musso and Morelli[13] cultivated from the blood/

blood and ascitic fluid of two cases of beri-beri a micrococcus which when injected into rabbits produced symptoms of beri-beri.

Scheube (1894) in his "Die Beriberi-Krankheit", while recognising the importance of the observations of Pekelharing and Winkler, takes great exception to many of them. He maintains that they did not produce beri-beri in the animals they injected and that the cocci found by them are not the cause, and not even one of the causes of the disease. He says if organisms are present in the blood, why, in 65 out of 80 cultures were no growths obtained? He says their results do not confirm each other and that some of them do not seem trustworthy. He does not think it probably that beri-beri can be produced by several organisms as was suggested by Pekelharing and Winkler, and again he thinks they treat the bacilli and yellow cocci they cultivated too lightly. He wants to know how these could get into the test tubes if not from the blood. He lays much stress on the liquifaction of gelatine, and indicates that the cocci which did liquify gelatine seemed more virulent and more certain in/

in their pathogenic characters than those that did not. He asks why no result was obtained by giving injections of the blood from beri-beris. He maintains that the cause of beri-beri has not yet been discovered.

Lastly, Fiebig[14] argues that the micrococcus cultivated by Pekelharing and Winkler is the same organism as Staphylococcus Pyogenes albus. He says the two have many resemblances, and that they have no differences which could not be explained by alterations produced by growth on certain culture media at certain temperatures.

From this account which I have given of the observations made, and the opinions held, as to the etiology of beri-beri by those who have been specially engaged in its investigation, one will readily appreciate that all are not agreed as to the nature of its specific micro-organism. And in this connection I may quote Dr. Spencer[15], who no later than a month ago, wrote as follows:-

"In spite of the arduous researches of Pekelharing Winkler and others, the organism which is the cause of beri-beri has not been satisfactorily or certainly determined."

At/

At this stage of the discussion therefore, all fresh observations which are carefully made and honestly recorded, cannot but be received as evidence of some value. And so I think I need urge no excuse for presenting the results of a bacteriological examination of the blood, in even only two cases of beri-beri.

II.

Shaki Shual, aet. 25, and Abdul Kadar, aet. 23, two firemen from the Anchor Line S.S. "Britannia", were admitted into Ward XI. of the Glasgow Royal Infirmary, on 26th. February 1896, complaining of being "affected with beri-beri". Their last port had been Blewfields, Nicaragua, and so far as could be made out they were two of seven lascars all affected with the same symptoms and presumably the same disease. Three of the seven had been sent to hospital in America, other two died on board the ship and the remaining two, our patients, came on to Glasgow.

When admitted into the ward each gave almost exactly the same clinical history and both showed very much the same physical signs. The symptoms they said were of three weeks duration and had set in with a feeling of great weakness, lassitude, and loss of power in the legs. The gait in each was unsteady, especially so in Abdual, who could not walk at all without support. The knee reflexes in both patients were absent, but the plantar and abdominal were quite active. No distinct muscular atrophy could be said to/

to be present, although there was certainly some loss of power in both the arms and in the legs. The reaction of degeneration was not fully developed. Most of the muscles in the limbs showed much lessened excitability to faradic electricity, yet some response was almost always obtained with a sufficiently strong current.

The patients complained of pains, and feelings of tingling and swelling in the feet, legs, and arms. The limbs were tender and painful to the touch and there was a sense of constriction at the ankles, knees and wrists, just as if a cord had been tied round these parts. Some degree of anaesthesia and analgesia was noticeable especially in the legs.

There was oedema of the legs, arms and trunk in both the patients, but it was much more marked in the case of Abdul who also had some cardiac complication. No murmur was to be heard over his precordial area but the sounds were feeble; and the second sound, and sometimes the first sound, was reduplicated. Abdul moreover, had signs of oedema at the base of both lungs and possibly/

possibly there was also some pleural effusion.

The spleen and liver were normal in both the cases, and in both there was a trace of albumen, and also sarcinae, in the urine.

An examination of the blood showed the following:-

Shaki - Haemoglobin 68% : Red Corpuscles 114%.

Abdul - Haemoglobin 76% : Red Corpuscles 12[illegible]%.

These two patients remained in hospital for two and a half months and their clinical history during that time is pretty much an account of their gradual recovery. Abdul had one bad attack of syncope four days after admission, and two days after that, each had a considerable rise of temperature, lasting two or three days, and for which no explanation could be found. Otherwise, the oedema gradually disappeared, the motor and sensory troubles passed off, and the patients were dismissed well.

During the residence of these patients in hospital I had the opportunity of examining their blood on four different occasions Viz:- March 6th, March 11th, April 2[illegible]th, May 9th; and the appearances which presented themselves were the same on each occasion and in each patient./

patient. The blood was examined with a 1/12th oil-immersion lens, both in the fresh state as well as in dried specimens stained with eosin and methylblue, with fuchsine, and with Biondi's stain. It was noted that the red blood corpuscles ran together into rouleaux, and the white were slightly increased in number, many of them containing large black granules. There also seemed to be an increase in the plates of Bizzozzero.

On addition to the above ordinary constituents of the blood, there were present small granules or micro-organisms in very active motion. These little bodies were seen as single "granules" or cocci, or as two cocci (diplococci), or more rarely as several cocci joined together. Sometimes as in the latter case, they were very similar to "rods", but this appearance I was convinced, was caused more by two or three cocci in a short chain than by a rod shaped organism. That the movements observed were not a brownian movement, but due to the organism itself, I am quite convinced; and on this point Dr. Middleton, Dr Steven and Dr. Workman, who were good enough to look at my preparations, were all agreed.

In the stained specimens the organisms appeared almost invariably as cocci in pairs, lying over, or attached to, the/

the red blood corpuscles. Never were any rod shaped organisms seen in these preparations. But it must be confessed that although I stained many specimens of the blood, the results obtained were not satisfactory; for the number of cocci in the stained preparations was never anything like so numerous as in those of fresh blood, and indeed they were sometimes very difficult to find. (slides Nos. 1 & 2.)

I next directed my attention to cultivating these organisms on artificial media. Tubes of agar-agar, serum-agar and gelatine were inoculated with the blood of our patients on the following dates and with the following results:-

March 7.

4 tubes of Agar-agar.
4 " " Serum-agar.
2 " " gelatine.

Growth appeared on two tubes only,— one agar-agar(growth A.) and one serum-agar(growth B.)— both from the blood of patient No. 1. (Shaki)

March 14.

4 tubes of agar-agar.
4 " " serum-agar.
2 " " gelatine.

The only growth here (growth C.) was on the gelatine

tube- from patient No. 2. (Abdul).
This tube had by mistake been placed in the incubator at 37 C. and so of course liquified.

April 20.

4 tubes of agar-agar.

4 tubes of serum-agar.

Growth appeared in two agar-agar (growths D.&E.) and one serum-agar tube (growth F.), all from case No. 2.

April 27.

6 tubes of agar-agar.

6 tubes of serum-agar.

Growth on one agar-agar tube (growth G) from case No. 1.

May 8.

6 tubes of agar-agar

6 " " serum-agar

4 " " gelatine.

Growth on one agar-agar tube (growthH) from case No. 1.

From the above it will be seen that of 56 tubes inoculated at various times from the blood of our two patients, unequivocal growths were obtained only in 8.

Of/

Of these eight tubes five were agar-agar, 2 serum-agar, and one gelatine (at 37° C.) Four of the growths were from patient No. 1, and four from patient No. 2.

Throughout this investigation every care was observed so that no organism from without should get into the tubes, special attention being given to the cleansing of the patient's finger from which the blood was drawn.

On further investigating the eight growths obtained from the blood, it was found that only three (E, F & H) were pure cultures. These three seemed to be the same organism for their characters were almost exactly alike. They grew rapidly on agar-agar there being in 24 hours (at 37° C.) an almost continuous growth along the streak. In from two to three days this had increased to an abundant pearly-white club-shaped growth with slightly scalloped edges (Fig. VI.), presenting an appearance almost identical with a similar growth of Staphyloccus pyogenes albus. Cover-glass preparations stained with fuchsine, showed the organisms to be cocci, grouped so as to form staphylococci. These also stained well with grain's method. (slides, Nos. 3. to 8)

Plabe/

'Plate cultures were made both with agar and with gelatine and these both showed the growths to be quite pure. With a low power the colonies on the agar plate were noted to consist of light-yellow or straw coloured discs, finely granular, with well defined edges and small dark centre. There were also other forms to be seen, much smaller, darker in colour, round or lozenge shaped, and in appearance rather like an uric acid crystal. These were usually separate from the larger discs but sometimes they overlapped them, seeming to grow out of them, giving an appearance as if the darker growths were the stalk, and the lighter growths the bell, of a mushroom-shaped colony. (Fig. 1.) On gelatine plates of five days growth, the colonies had much the same appearance as the above larger discs, only they seemed thicker and more granular. There were very few of the dark uric acid-looking plaques to be seen. (Fig. 2.)

On examining a hanging drop preparation, cocci solitary and in pairs, were seen to be in very active motion. The appearances here reminded one of the appearance these organisms presented in the fresh blood; the kind of movement was almost exactly alike in both cases and the movement certainly was not a movement in the/

in the fluid, but inherent in the cocci themselves. As to whether or not these cocci had flagella I cannot say definitely, but if they had I was quite unsuccessful in my attempts to stain them.

The micrococci grow rapidly in Bouillon Broth giving in 24 hours a muddy appearance. The reaction of this culture medium after a few days was strongly acid. It was also demonstrated that a small amount of sulphuretted hydrogen was given off from this medium during the growth of the organisms. The addition of potassium nitrite and sulphuric acid to the Bouillon Broth, showed the indol reaction though faintly, yet quite distinctly.

When grown on milk a coagulation is developed in from two to three days and the fluid which separates has a strongly acid reaction and a distinctly sour smell. This fluid added to solid gelatine does not liquify the gelatine.

b Stab-cultures in gelatine develop slowly and liquifaction does not seem to be constant. When it does take place it does so very slowly sometimes along the streak, sometimes transversely at the upper part of the gelatine (Fig. 7 & 8). Each of the three cultures we are now considering at first did liquify gelatine but growing them on gelatine seemed to lessen and even destroy this capacity.

The growths E & F. are examples of this, for now after seven or eight months growth partly on gelatine and partly on agar, I cannot get them to liquify gelatine. At no time, even in summer, did liquifaction take place in less than a fortnight and sometimes it did not develop till six weeks had elapsed. In the tubes where liquifaction took place the organisms, which fell to the lower part of the fluid, developed a dirty orange-colour pigment. The fluid itself had a neutral reaction.

The remaining five growths A, B, C, D, & G, were impure but on making plate cultures and isolating their componant organisms, it was found that each of the five contained a staphylococcus similar to that of E, F, & H, whose characters we have just been considering. From each there was in addition isolated a rod-shaped organism, but not in any two of the growths was this rod the same.

Thus in growth A, where both cocci and rods are to be made out in the cover glass preparation (slide No. 9), a staphylococcus (slide No. 10) was isolated, whose appearances on plate cultures, in agar tube, in Bouillon Broth, in its power of liquifying gelatine etc./

etc., showed it to be the same as the growths E, F, & G. The other organism, on agar plate cultures formed light brown or yellow colonies, quite round, granular in appearance, with fairly well defined edges. There were also some plaques to be seen, light yellow in colour, and usually overlapping each other (Fig. 3). The appearances on gelatine plates were much similar. Growth was seen on an agar tube in 24 hours, as pearl-white rounded colonies, tending to run into each other along the streak. On staining this growth it was seen to consist of thin short rods, not staining uniformly. These stain well with Gram's method but every attempt to stain spores was unsuccessful (slides 11 & 12). Hanging drop preparations showed rods in active motion. Gelatine liquifies slowly.

Growth B. also contained a staphylococcus of the type I have already described except that it never liquified gelatine (slide 14). The rod organism isolated had very distinctive characters. On agar plates a colony appeared, under a low power, as a more or less rounded body of amber colour, with several processes, giving in all an appearance as like as possible/

possible, to that of the itch insect — acarus scabiei (Fig. 4). After four or five days growth on agar tube, the culture which was white, began to spread out at its edges into branches, presenting an appearance like that of a fir-tree (Fig. 9). Cover-glass preparation showed short thick rods, many of which only stained at their two ends. These rods stain well with Gram's method and show spore formation when stained for spores (Slides 15. 16 & 17). A hanging drop reveals large strong rods all in active motion. Growth in gelatine stab-cultures is very slow and when it does take place, it is in the form of white fluffy discs deep down in the culture medium. There is no liquifaction of the gelatine.

The next growth, C, contained a staphylococcus the same as in the other tubes (slide 19). This organism grew well in gelatine but did not cause liquifaction. The other growth isolated from tube C, was a rapidly growing bacillus which readily liquified gelatine, and in its plate culture appearances and other characters, showed itself to be BACILLUS MESENTERICUS VULGATUS (Slides 20, 21 & 22).

Growth/

Growth D. (Slide 23) also contained the white staphylococcus which in this case liquified gelatine in three weeks. The other organism present here was a bacillus, which on agar plates presented darkish-brown, hairy-looking colonies, with rather irregular outline (Fig. 4). The agar tube taken from this, in two days had a thin skum-like growth over part of its surface, and on being stained it was seen to consist of long strong rods somewhat swollen at their ends (Slides 26 & 27). They stained by Gram's method and in the hanging drop preparation were seen to be actively motile. The growth on the agar tube was slow and not at all abundant as was also the case in the gelatine stab-culture. The gelatine did not liquify. Spores were made out to be present.

In the last growth we have to consider — growth G. (slide 28) — the staphylococcus present did not liquify gelatine but otherwise corresponded in every detail to the type so often mentioned. The rod-form here consisted of short bacilli which stained with Gram. But it was very difficult to cultivate either on agar or/

or on gelatine, and as it died soon its characters were not completely made out(Slide 31).

We have now to consider the pathogenic characters of these growths from the blood of our beri-beri patients. So that I shall proceed to give an account of the results obtained by injecting 6 rabbits with some of these organisms.

The plan pursued in every case was to grow the micro-organism in Bouillon Broth for 48 hours, and then to inject a certain quantity of this culture, either full strength or deluted with an equal quantity of sterilised water, into the abdominal cavity of the rabbit. Special care was taken in every case to make the syringe employed aseptic, and to cleanse thoroughly the skin of the rabbit before giving the injection.

Rabbit I, a fairly large animal, was injected with the pure staphylococcus growth E.

11 injections were given in 26 days i.e. three injections per week. The first five injections each consisted of 3 c.c. of the Broth culture, the sixth of 4 c.c., seventh and eighth of 5 c.c., and the ninth, tenth, and eleventh, each of 12 c.c.,

The day after the last injection the rabbit showed/

showed signs of paralysis. It could not stand but lay on its side panting for breath. The fore legs and hind legs were extended and it seemed quite unable to draw them up. The animal was killed at once and an examination made a few minutes later; but nothing abnormal could be made out in any of the organs. There was very slight induration at the part of the abdominal wall where the injectionshad been given, but no appearance of effused lymph or inflammation of any of the peritoneal membranes. Liver, kidneys and spleen seemed quite healthy. The nerves of the hind legs were kept for microscopic examination.

Cultures were taken from the blood in the heart, in the spleen and from the liver. Eighteen tubes in all were inoculated and in exactly half of these was a growth obtained. Cover-glass preparations were made, and in every tube the organism present was found to be a staphylococcus. Four of the tubes were examined in detail and these were all demonstrated to have the same characters as the coccus which had been originally injected into the rabbit (Slide No. 32)

Rabbit 2

Rabbit 2, A small rabbit, injected with staphylococcus growth F.

In this case 5 injections each of 2·5 c.c. of a Broth culture, were given in 10 days. On the twelfth day the animal was seen to be dying, its head being much retracted and the hind legs fully extended. The rabbit was killed and examined at once. The appearances which now presented themselves were exactly the same as in rabbit 1, except that on the surface of the liver there were several small white spots, about the size of half a millet seed, and which must have been small abscesses. They contained many staphylococci. Tubes were inoculated from these abscesses and with the blood of the heart and spleen. There was a white growth in the heart culture and in the abscess culture, and both of these were shown to have the characters of the white staphylococcus which had been injected into the rabbit (slide 33).

Rabbit 3, also a small animal, was injected with growth H.

Three injections of the Bouillon culture each of 6 c.c. were given in five days. About a quarter of an hour/

hour after the last injection the animal was found dead. It had been noted before giving this injection that the animal seemed ill. However, it could sit up quite well, although it made practically no movement and gave almost no resistance when one was injecting the fluid.

The post-mortem here was quite negative at least as regards naked eye appearances. There was no sign whatever of any inflammatory process in the abdominal cavity. Owing to an accident no growths could be obtained from the blood of this animal.

Rabbit 4, a much larger animal than the last, was injected with the same growth H. In this case it took six injections each of 3 c.c., extended over 12 days, to kill the rabbit. Although it had seemed quite well at the last injection, the rabbit was found dead the next morning. The post-mortem appearances corresponded to those of rabbit 2 in their being several abscesses or collections of cocci scattered over the surface of the liver. Otherwise the macroscopic examination was negative.

A growth was obtained in only two of the cultures taken and/

and both of these were from an abscess in the liver. In this rabbit however only eight tubes in all were inoculated and this may quite well account for there being no cocci grown from the blood. The two growths from the liver were quite pure and contained the white staphylococcus, the same as had been injected into the abdominal cavity (Slide 34).

Rabbit V. was small, simidar to rabbit 2. It was injected with growth A. which contained both white staphylococci and bacilli.

Five injections of 2·5 c.c. were given in 10 days. The rabbit died on the twelfth day with limbs extended and head retracted. The post-mortem appearances were exactly similar to those in the last rabbit, there being here also small white abscesses in the liver. White staphylococci were grown from the blood of the heart and spleen but no bacilli were found in any of the cultures (Slide 35).

Rabbit VI. was injected with growth G.— both cocci and bacilli.

Four injections each of 6c.c. were given in 8 days. Five hours after the last the animal died with well marked signs of paralysis. The post-mortem was negative;

no/

no abscesses were present in the liver. Four tubes were inoculated, two from the blood in the heart and two from the spleen. Growth presented itself in three of these. Two were pure staphylococci; (slide 36) but the third in addition to the same staphylococcus, contained a rod-shaped organism(slide 37). This bacillus however, on plate culture and otherwise, showed itself to be quite a different organism from that contained in growth G, and so I take it to be an impurity, and to have no relation to the subject in hand.

Nerves from the hind legs of these six rabbits were removed, stained and examined miscroscopically. In doing this great care was taken so as in no way to stretch or otherwise bring about an artificial appearance in any of the nerve fibres.

The nerves were fixed and stained at the same time, in osmic acid (1%), and most of them counter-stained with alum-carmine. Portions of the nerves were next cleared in a mixture of aniline oil and xylol, and then in xylol alone; and while in the latter the individual nerve fibres were as much as possible separated the one from the/

the other. The specimens were mounted in Canada Balsam.

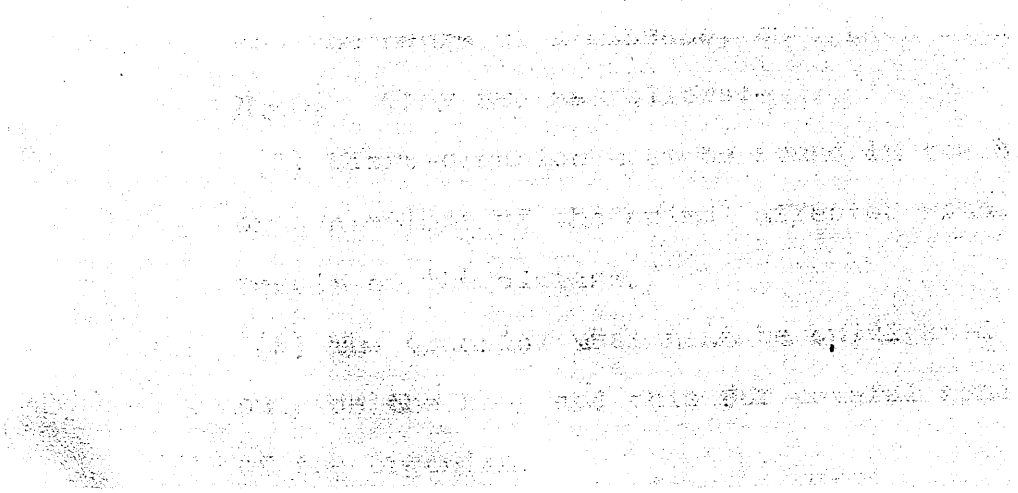
To make sure that this process had nothing to do with the altered appearance in the nerves under consideration, I prepared in exactly the same manner, nerves from a healthy rabbit, and found them to remain perfectly normal.

A careful microscopic examination of the nerves from our six rabbits readily convinces one that a parenchymatous degeneration is present in every case (slides Nos. 38 to 43) This certainly is much more marked with some than with others.

For example in Rabbits 2, 3, 5, & 6, the degeneration is so complete that there is difficulty in finding a normal nerve fibre. In cases 1, & 4, on the other hand, many healthy fibres are to be seen.

In the degenerated fibres the myelin is sometimes seen as a series of frothy-looking masses filling up the sheath and giving a segmented appearance to the fibre. Sometimes the myelin is in the form of small rounded balls, stained black or brown, in the centre of the sheath. Sometimes again, the sheath seems empty except/

empty except for its nuclei which in some of the specimens are seen to be much increased in number. Some of the nerve fibres have a spindle shape, one part of the sheath being filled and swollen up with frothy myelin, while an adjoining portion has its walls in close apposition, the myelin at this part having disappeared.



III.

The importance of micro-organisms as factors in the causation of disease is now well recognised. But so often has been claimed for a certain disease, the discovery of its "specific microbe" and so often in turn has this claim been found to be false, that the scientific world is very slow to recognise any such discovery without its being backed by the strongest proof.

The conditions which are now considered necessary to prove that a certain micro-organism is pathogenic and the cause of a disease, have been laid down by Koch. They are as follows:-

(1) Micro-organism must be found in the blood, tissues, or secretions of the animal affected with, or dead the result of the disease.

(2) The organism must next be cultivated on media outside the body and this for several generations of the organism.

(3) This culture in turn, must be shown to produce on inoculation, the same disease in a healthy animal.

(4) In/

(4) In this animal the same micro-organism must again be found .

To these four conditions have been added (5), that the same micro-organism must be found in all cases of the particular disease under consideration and (6), that this organism must never be found in any other disease.

The first four conditions were certainly fulfilled by Pekelharing and Winkler before they claimed their white staphylococcus to be the specific micro-organism in beri-beri. And in view of all the criticism their observations and conclusions evoked, it is a great satisfaction to me to find that my results, be they only from two cases, correspond to, and if I may say corroborate, in almost every detail, the work of these two most distinguished men.

I found a micrococcus to exist in the blood of our two patients on every examination. A micrococcus was demonstrated to be present in all the cultures obtained from the blood. These cultures injected into rabbits were followed by signs of paralysis, and, at the post-mortem examination Peripheral neuritis — the characteristic lesion of beri-beri/

beri-beri—was a constant appearance. Lastly, the same micrococcus was found in the blood or tissues of the rabbits so treated. So that I too, have fulfilled Koch's four postulates and indeed the fifth, for the same organism was present in all cases as well as in all the cultures examined. And therefore my conclusion also must be that this white staphylococcus is the cause of beri-beri.

On considering again for a moment the opinions of the former writers on this subject, we find that the great majority of them also seems to be in favour of the micro-organism being a white staphylococcus. Pereira found micrococci in active motion in the blood. And the reason doubtless that he failed to obtain results with injections of these micro-organisms, is that he did not understand, that not one only but many injections, are required to develope the disease. His observation as to the presence of these micrococci in 2½% of healthy people corresponds in a measure to that of Pekelharing and Winkler who as we have seen, explain that in an affected district the so called healthy are seldom really healthy, but suffer in some/

some degree from the presence of the micrococci in their circulation. The later observations too, of De Lacerda, the conclusions of Van Eecke, of Leopold, of Musso and Morelli, all are in favour of a micrococcus. It is true that much from these last cannot be taken as sound evidence, for they have not in many cases fulfilled Koch's "Laws" before making their conclusions. Yet be its value what it may, this evidence is in favour, and certainly not against a micrococcus being the specific cause of beri-beri.

Ogata on the other hand, claimed a bacillus to be the organism. This however as far as I can make out, was never cultivated from the blood of his patients but from the alimentary canal, and after death from the fluids or tissues of the body. Now one understands that it would not be difficult to cultivate from a dead body, and this is especially in a warm climate, an organism whose toxins, injected into a mouse, would cause the "symptoms of beri-beri". And may not the same be said of a culture taken from the alimentary tract, especially in patients not in good health? I think therefore we need give this evidence of Ogata in favour of a bacillus, little consideration.

As/

As to explaining the presence of the bacilli found by me in five cultures, and by Pekelharing and Winkler in their cultures and so often in the fresh blood, considerable difficulty presents itself. That none of the bacilli in my five growths is likely to be the cause of beri-beri seems pretty certain. In the first place they were never seen in the blood either in fresh or in stained specimens. They are all different organisms. One of them, Bacillus Mesentericus, has well known properties of its own. Two of them, each associated with the white staphylococcus, on being injected into rabbits, could not be found in their circulation, and therefore they probably did not live in the blood of the rabbits. Certainly to have completed the argument I should have tested the pathogenic character of pure cultures of these bacilli. But as no one of them occurred twice, their presence did not seem of sufficient importance to necessitate this proceeding. And here it is to be remembered that Pekelharing and Winkler proved the non-pathogenic character of the bacilli they isolated.

What/

What then is the meaning of these bacilli in growths taken from the blood and how do they get there? They must either have been in the blood, on the skin of the finger, or have otherwise got into the tube from without during the process of inoculation. If from either of the two latter sources, why have they only grown when associated with a staphylococcus, an organism almost certainly grown from the blood, and why never in any of the 48 tubes that remained sterile? If again they are from the air why only in one case—bacillus Mesentericus—was it a well known micro-organism?

The remaining explanation which presents itself is that the bacilli exist in the circulation as saprophytes. And in favour of this view it might be pointed out that the presence of the staphylococcus of beri-beri must lower the vitality and resisting power of the blood and tissues. In this condition therefore would be possible the existance and growth of saprophytes. That saprophytes however are not common in the blood in disease I can affirm, for during the past two years I have examined the blood both in stained as well as in fresh specimens, of some

80 patients, chosen from the general wards of the hospital. Only in very few cases, some pneumonias and cases of endocarditis, were micro-organisms of any kind to be found. But on the other hand, it is well known that certain pathogenic organisms have not uncommonly present with them others which are non-pathogenic. As an example of this one may cite the frequent association in pus of the micrococci cereus albus and flavus, with the streptococci and staphylococci pyogenes.

But even if these bacilli in our cultures were not saprophytes but had well known pathogenic characters, that in no way need be an argument against our white micrococcus being the specific micro-organism of beri-beri. The presence of staphylococci and streptococci in diphtheria, associated with the bacillus of diphtheria, does not lessen the claim of that bacillus to be the specific cause of the disease; even although it has been shown that the toxins from the cocci when injected into guinea pigs caused paralysis.[18] The important thing is, that in diphtheria the bacillus of diphtheria is found constantly present while the cocci are not; and the bacillus too, is known to produce diphtheria/

diphtheria whenever injected into animals.

What I wish to bring out therefore in regard to beri-beri, is that the great mass of evidence so far seems to be in favour of the micrococcus of Pekelharing and Winkler being present in all cases of beri-beri. And as this micro-organism had been shown to have the property of producing, when injected into animals, an neuritis similar to that in beri-beri, the conclusion to be drawn is that this micrococcus is the cause of beri-beri.

That the bacilli and cocci seem in the blood by the Dutch writers, and that the suggestion by them that these are just two different forms of the same organsim, — is an explantion which does not commend itself to one's mind. For as far as I know there is in Bacteriology, no analogy for such explantion. It is much more likely that the Dutchman were dealing with impure growths, containing both cocci and bacilli, and which as I found, often required repeated plate cultures to be made before each could be isolated.

As regards the variety of micro-organism found in the blood by Leopold and Van Eecke, their descriptions are/

are not sufficiently definite to let one recognise them, nor is the account of the pathogenic properties given by Van Eecke of his, at all satisfactory. Besides, most of what has already been said on the bacilli found in the blood, will also apply to these organisms. And one more point I wish to note here, is that in my two cases, the micrococci did not completely disappear from the blood on the patients leaving the place of their infection. For these micro-organisms were still to be seen and were cultivated after two and a half month's residence in hospital, and after practically all signs of the disease had disappeared.

We must now consider Scheube's critism of the Dutch writers. Much that he says is doubtless small and petty and requires no answer, but there are some points raised by him which may well demand our consideration.

(a) Scheube asks why are the micrococci of beri-beri so difficult to cultivate from the blood; Pekelharing and Winkler only obtaining 15 growths out of 80 inoculations, and not all of these containing cocci.— I do not know that any very satisfactory answer can be given to this question otherwise than that they are difficult to grow. And I think that this does not apply to beri-beri alone, but also to the micro-organisms found in the blood in other diseases where the difficulty

difficulty is often quite as great. This is the only explanation I can give for the negative results obtained by Sternberg and other observers.

(b) He thinks they treat too lightly the yellow micrococci and he wants to know how these get into the cultures.— To this I can add nothing more than I have already said on the subject.

(c) He asks why injections with the blood of beri-beri patients does not cause the disease in animals.— The reason doubtless is that up to a certain point the blood has the capacity of destroying micro-organism, either by its phagocytes or by an "anti-toxin" which it seems to contain in its serum. When too many organisms get into the circulation, the blood is not able to deal with them, and so they grow producing their toxin, which being no longer neutralised, gives rise to the symptoms of the disease.

(d) Scheube seems to consider it impossible that the Pekelharing and Winkler staphylococcus may at one time liquify gelatine and at another not be able to do so.— But is it not the case that such a well known micro-organism as the Staphylococcus Pyogenes Albus sometimes shows this character? And the observations extending/

extending over ten months, which I have made on this point, entirely confirm those of the Dutch writers. I found the liquifaction of gelatine to be always slow.
I found that some of the growths which liquified gelatine at first did not do so later on. This as far as I could make out was in those organisms which had been growing for a considerable time on gelatine.
Three of the growths at no time liquified gelatine; but these were separated from impure growths, and possibly the presence of the other organisms had lessened the virulence of our micrococcus. As far as I could make out the virulence of the micrococcus was in direct proportion to its power of liquifying. For example growth H always liquified gelatine and when injected into rabbits it killed them rapidly and with small doses (rabbits 3 & 4). GrowthE on the other hand, which had lost the power of liquifying gelatine before being injected into rabbit 1, did not kill for many days more, and even then required much larger doses of the culture. Nor did the cultures grown from these and the other rabbits inoculated, liquify/

liquify gelatine any more rapidly. This seems strange for one expects the virulence of an organism to be increased on passing it through an animal that is not immune.

I have yet to refer to Hiebig's objection to the staphylococcus of Pekelharing and Winkler. He says it has the same characters as Staphylococcus Pyogenes Albus and he maintains that it is the same organism. It certainly has much the same morphological appearances but the pathogenic characters of the two are very different. For would a culture of the Staphylococcus Pyogenes Albus injected into the abdominal cavity of a rabbit, produce no sceptic result? In not one of my six rabbits did an abscess form as the seat of inoculation and in not one was any inflammatory condition of the Peritoneum to be made out. Certainly in some of the rabbits minute abscesses were found in the liver, but these I take it, may be looked upon as collections of cocci growing in a part of the circulation where the blood tends to stagnate. As to whether the Staphylococcus Pyogenes Albus/

Albus may under certain circumstances become the staphyloccus of beri-beri, or vice versa, I cannot say; but at present there seems to be as much difference between the two as there is between the pseudo-bacillus of diphtheria, and a bacillus of diphtheria itself.

And now in conclusion It may be asked—Is it finally settled that the staphyloccus of Pekelharing and Winkler is the specific micro-organism in beri-beri? The reply is difficult. We know that medicine is not an exact science, and that its conclusions cannot therefore be drawn with the absolute certainty of a proposition in Euclid. The methods of medicine are those of observation and induction, and only when very numerous observations have been made, can the conclusion be drawn with even a degree of finality. But where a finding cannot be absolute it may sometimes be comparative: when our observations are only few then our conclusions can only be comparatively true. And in medicine this latter position is one we often find ourselves occupy, and it would seem to be the one we are now standing in as regards the causation of beri-beri. A certain number of data have been furnished/

furnished as to the etiology of this disease, and from these the conclusion to be drawn is as I have contended in the foregoing pages, that the staphylococcus of Pekelharing and Winkler is its specific micro-organism. This doubtless is only a "comparative" conclusion for we have not yet data to draw one more certain. Many more observations are required, and it is with this in view that I have presented mine. For them I have to say, that if they add only in a very slight degree, to the building up of our knowledge of this disease, it will be to me an endless satisfaction.

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MICROSCOPIC SPECIMENS.

No.1. BLOOD. Fixed with heat and alcohol and ether.
stained with fuchsine.
Shows cocci, mostly in pairs and attached to the red blood corpuscles.

No.2. The same as No. 1., but fixed with heat only.
Here the red blood corpuscles do not stained, but only the nuclei of the white.

No.3. GROWTH E. Stained with fuchsine.
Staphylococci

No. 4. Same as No.3. stained with Gram.

No.5. GROWTH F. Stained with fuchsine.
Staphylococci.

No.6. Same as No.5 stained with Gram.

No.7. GROWTH H. Stained with fuchsine.
Staphylococci.

No.8. Same as No.7. Stained with Gram.

No. 9. GROWTH A. Stained with fuchsine.
shows both rods and staphylococci.

No.10. GROWTH A_1 Stained with fuchsine.
Staphylococci pure.

No.11. GROWTH A_2 Stained with fuchsine
thin slender rods not uniformly stained.

No.12. Same as No. 11 stained with Gram.

No.13. GROWTH B Stained with fuchsine.
contains rods and cocci.

No.14. GROWTH B_1 Stained with fuchsine.
Staphylococci pure.

No.15. GROWTH B_2 Stained with fuchsine.
Bacilli only.

No.16. Same as No.15 Stained with Gram.

No. 17. Same as No. 15. Double stained for spores.

No.18. GROWTH C. Stained with fuchsine
contains both rods and cocci.

NO. 19. GROWTH C_1 Stained with fuchsine.
Staphylococci pure.

NO. 20. GROWTH C_2 Stained with fuchsine.
Bacilli only.

NO. 21. The same as No.20. Stained with Gram.

NO. 22. The same as NO. 20. Double stained for spores.

NO. 23. GROWTH D. Stained with fuchsine.
contains rods and cocci.

NO. 24. GROWTH. D_1 Stained with fuchsine.
Staphylococci pure.

NO. 25. The same as No.24. Stained with Gram.

NO. 26. GROWTH D_2 Stained with Fuchsine.
Bacilli only.

NO. 27. The same as NO. 26. Stained with Gram.

NO. 28. GROWTH. G. Stained with Fuchsine.
contains both rods and cocci.

NO. 29. Growth. G_1 Stained with Fuchsine.
Staphylococci only.

NO. 30. The same as No.29. Stained with Gram.

NO. 31. GROWTH G_2 Stained with fuchsine.
Bacilli.

NO. 32. Growth from blood of rabbit 1. fuchsine.
Staphylococci.

NO. 33. Growth from blood of rabbit 2. fuchsine & Gram.
Staphylococci.

NO. 34. Growth from blood of rabbit 4. fuchsine.
Staphylococci.

NO. 35. Growth from blood of rabbit 5. fuchsine & Gram.
Staphylococci.

NO. 36. Growth from blood of rabbit 6. fuchsine.
Staphylococci.

NO. 37. Growth from blood of rabbit 6. fuchsine & Gram.
Bacilli.

NO. 38 to 41. Nerves from rabbit 1. Stained with osmic acid.
Many of the nerve fibres quite normal.
Many others where the Myelin inside the sheath is inglobules. In some fibres no Myelin remains.

NOS. 42 & 43. Nerves from rabbit 2. Stained with osmic acid.

Neuritis well marked, few fibres seen to have escaped. The frothy swelling or coagulation well shown. In places increase of nuclei.

NOS. 44 to 47. Nerves from rabbit 3. Stained with osmic. acid. also show well marked degeneration. In many places the Myelin has entirely disappeared from the sheath.

NOS. 48 to 51. Nerves from rabbit 4. Osmic Acid. nerve degeneration not so well shown as in rabbits 2. & 3. It is however quite distinct.

NOS. 52 & 53. Nerves from rabbit 5. Osmic acid. degeneration well marked.

NOS. 54 to 55. Nerves from rabbit No. 6. Osmic Acid. degeneration well marked.

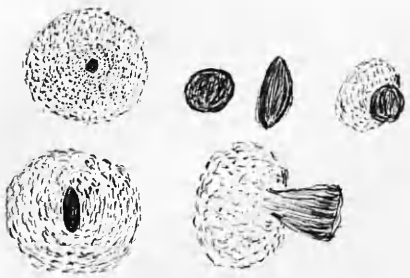

Fig. I.

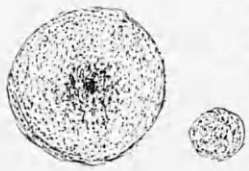

Fig. II

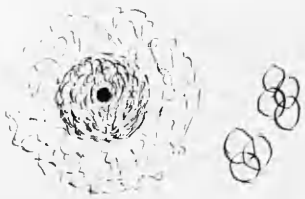

Fig. III

Fig. IV

Fig. V

Fig. VI

Fig VII

Fig VIII

Fig. IX.

Fig I. Growths E, F, G. Colonies on agar plate.

Fig. II. The same on gelatine plate.

Fig. III. Growth A_2. Colonies on agar plate.

Fig. IV. Growth B_2. Colonies on agar plate.

Fig. V. Growth D_2. Colony on agar plate.

Fig. VI. Growths E, F & G. Streak culture on agar tube.

Fig. VII } The same. Stab-culture in gelatine.
Fig. VIII }

Fig IX. Growth B_2. Streak culture on agar tube.

W. K. H.