Observations on the Brains of Men and Animals infected with Various Forms of Trypanosomes. Preliminary Note.

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The material upon which these observations are based has been forwarded to me from Uganda, with the exception of the brain of a rabbit kindly given to me by Dr. Plimmer.

By the desire of the Committee on Tropical Diseases of this Society, Colonel Bruce has given instructions to his assistants, and they have forwarded to me from Entebbe, material from cases of Sleeping Sickness, 24 in all; also portions of the brains of eight monkeys experimentally infected, two oxen, and one donkey.

The tissues have been preserved in Formol-Müller's solution, or they have been sent already embedded in paraffin after having been hardened a short time in formalin. Sections were cut of either 5 μ or 10 μ in thickness; they were stained by Romanowsky, Leishman, Nissl polychrome, or Weigert-Gram methods, thus enabling observations to be made regarding:—

(*a*) The existence of trypanosomes or Leishman bodies.

(*b*) Changes in the ganglion cells and neuroglia cells.

(*c*) Changes in the blood, the endothelial cells of the vessels, and the peri-vascular spaces, and soft membranes.

(*d*) The existence of micro-organisms.

(*e*) The existence of plasma cells, and other cells indicative of chronic inflammatory degenerative changes.

It may be mentioned that in a number of instances sections of the lymphatic glands, some of which were removed during life and others *post-mortem*, were examined by the same methods. The principal pathological conditions observed were either drawn or photographed.

In every case of Sleeping Sickness, in which there were symptoms during life, the characteristic change (which I described in two cases reported by Sir Patrick Manson) of a chronic meningo-encephalitis was found. In every chronic case I have found also plasma cells of Marscholko, which by some observers were considered to be pathognomonic of another chronic meningo-encephalitis, general paralysis of the insane.

Every stage of the development of these plasma cells from lymphocytes can be observed in the brain tissues, also in the chronically inflamed lymphatic glands. Likewise, in chronic cases, morular cells, indicative of chronic inflammation, can be seen in most cases.

From the clinical notes furnished in these cases, it is apparent that there is a correlation between the severity of the symptoms, the chronicity of the disease and the degree and intensity of the chronic inflammatory process, as evidenced by the abundance of lymphocytes in the peri-vascular spaces and in the subarachnoid space, also by the number of plasma cells and morular cells.

With regard to the ganglion cells, the very chronic cases show a very marked degeneration of the ganglion cells of the central nervous system, particularly of the medulla oblongata and the cortex—proportional to the degree of affection of the vessels—many of the smaller of which are completely occluded, partly by the accumulation of lymphocytes, partly by the proliferation of the nuclei of the endothelial cells. Many of the capillaries are completely occluded by this process, and the result is not merely a chromatolysis of the ganglion cells, but a coagulation necrosis and destruction. Where there is this advanced degeneration, there is a marked proliferation of the glia cells, and a large number of spider cells can be seen; in fact, the appearances, as the accompanying pictures show, closely resemble, in many respects, the chronic inflammatory changes met with in general paralysis. Except that, whereas in the latter disease, the vascular change is in great measure secondary to the degenerative change of the ganglion cells; in Sleeping Sickness the chronic inflammatory process is universal throughout the central nervous system, and the ganglion cells are destroyed, secondarily to the occlusion of vessels.

The glia cell proliferation is not nearly so pronounced in Sleeping Sickness, because there is so much less wasting of the brain substance. In one case, so marked was the peri-vascular infiltration in the grey matter of the cortex, that after hardening in Müller's solution the vessels could be seen with the naked eye as glistening pearly lines and points.

In the blood contained in the vessels of a few of the chronic cases without micro-organismal infection, a solitary trypanosome or a portion of a trypanosome could very occasionally be seen, but it is a remarkable fact how very seldom, in the immense number of sections examined, I have been able to find evidence of trypanosome infection by examination of the blood contained in the vessels; I have, therefore, concluded that these organisms cannot be abundant, and that if they produce this chronic inflammation as all the facts in the etiology of the disease prove, it may either be that they

(1) produce a toxin which acts as the irritant; (2) they *undergo morphological changes* in the blood or cerebro-spinal fluid; or (3) that the secondary or terminal infection with which nearly all these cases were affected (except three), with diplococci, diplo-streptococci, or occasionally other organisms such as cocci, Friedlander's bacillus and bacillus coli, leads to the destruction of the trypanosomes.

It is probable that the defences of the organism against bacterial invasion are lowered by the trypanosome blood infection. It has been shown that recently a large number of natives have been dying of pneumonia. The diplococcus is one of the most prevalent organisms found in the body. Again, negroes, owing to jiggers and other sources of infection by pyogenic organisms have therefore ready to hand a source of secondary or terminal infection.

A very interesting case in this respect was Bara Risgalli; this man for a long time had infection with *Trypanosoma Gambiense* in the blood; these organisms were also obtained from his lymphatic glands by Captain Greig. Sections of the glands removed during life were examined by me, and I found evidence of degenerated trypanosomes, macro-nuclei and micro-nuclei; also the glands showed marked evidence of chronic inflammatory change; plasma cells and degenerated cells being abundant. The gland removed during life was sterile, that is free from micro-organisms, and no diplococci could be discovered in the sections which I examined. At this time, he showed no signs of Sleeping Sickness; later he was taken ill, and as I learnt from Captain Greig, he died in 10 days of pneumonia with cerebral symptoms.

Examination of the brain showed a well marked acute pneumococcic meningitis; in fact, I should not have thought of Sleeping Sickness upon looking at the sections without prejudice, for the leucocytic infiltration was almost entirely confined to the membranes, consisted almost entirely of polymorphonuclears, and it did not extend into the peri-vascular spaces. Amid and within the leucocytes were immense numbers of diplococci with capsules; the lymphatic glands, which were previously sterile, now all contained diplococci. Whether this man would in time have developed the chronic meningo-encephalitis of Sleeping Sickness and its associated progressive phenomena, I am unable to say; but it is an interesting point in connection with the fact that some authorities look upon the *Trypanosoma Gambiense* as a distinct form from that which produces Sleeping Sickness.

The European case, reported by Sir Patrick Manson, of a missionary's wife who died in England with the lesion of Sleeping Sickness, having suffered for some time with trypanosome fever, and with *Trypanosoma Gambiense* in the blood, is opposed to the view of a distinct organism. It is possible,

therefore, that Bara Risgalli, had he lived longer, would have developed Sleeping Sickness, for in some places a few of the vessels showed slight lymphocyte proliferation in the surrounding lymph spaces; but even this is not conclusive evidence, for I have found the same in chronic diplo-streptococcic meningitis.

There is another man, Tabula, whose glands, removed *intra vitam*, I have examined, and which showed exactly the same changes as Bara Risgalli, and who, I understand, has similar trypanosomes in the blood, and general glandular enlargement, but has not yet developed any signs of Sleeping Sickness. It will be interesting to see what becomes of him.

It has been shown that the cerebro-spinal fluid in Sleeping Sickness always contains trypanosomes, and likewise the juice of the lymphatic glands by puncture during life. On examination of *sections* of the glands in a number of these cases, in which active trypanosomes had been found during life, I observed only rarely a body which I could definitely call a trypanosome, therefore, it is not surprising that I was unable to find, after very careful search of many hundreds of sections, any body which I could definitely recognise as a trypanosome in the meningeal peri-vascular cell infiltration of the central nervous system. Yet, as the coloured drawings show, not only did one see similar cells and products of chronic inflammatory change in the peri-vascular lymph spaces, but also similar products of degeneration and similar staining chromatin bodies and bits like those seen in the lymphatic glands, which we have reason to believe may be products of degenerated trypanosomes. Moreover, I have occasionally seen a macro-nucleus with its accompanying micro-nucleus amidst the cells of the peri-vascular infiltration. In the lymphocytes themselves, in a chronic case in which I could discover no diplococcal infection by Gram's method, I have found deep staining bodies, oval or round in shape, but I am unable to affirm what they are.

If the trypanosomes are continually being destroyed, as they seem to be by the cells, it is not surprising that more evidence of their existence is not seen. It is remarkable in transections of the blood vessels in very chronic cases, to observe how few are the polymorpho-nuclear leucocytes, and how numerous the small and large mono-nuclears, and apparently these get into the peri-vascular spaces.

Examination of tissues of other organs from cases of Sir Patrick Manson's showed that not only the brain and glands are affected, but serous membranes and organs of the body, by this lymphocyte infiltration around the vessels, although to a much less degree.

Examination of the Brains of Animals Infected with Trypanosomes.

It has been stated that trypanosomes cannot be shown in sections of the brain, and that the hardening fluid may have been the reason why more definite evidence of the trypanosome infection in sleeping sickness has not been observed. The following observations show that both these hypotheses are probably untenable.

(1) The brain of a rabbit inoculated with *Surra*, which died three months later, hardened in formol, was kindly given me by Dr. Plimmer, and showed the following appearances in sections. By any of the staining methods employed, nearly all the blood vessels showed masses of trypanosomes, as the coloured drawings exhibit. Single trypanosomes could be seen in the capillaries; in the larger vessels solitary trypanosomes, and whorls of trypanosomes, and plasmoidal masses, which may either be degenerated trypanosomes consisting of a zooglœal mass, in which more deeply stained macro-nuclei and micro-nuclei can be seen, or, as Plimmer and Bradford consider, of amœboid forms. But in spite of this extraordinary trypanosome infection, the blood vessels showed little or no inflammatory reaction. The peri-vascular spaces showed no lymphocytes, the ganglion cells showed marked chromolytic changes, otherwise there was nothing noteworthy in the nervous system.

(2) The brains of two oxen infected with Jinga trypanosomes were examined. The animals died within three months of infection; the results of the examinations were extremely interesting and will be given in some detail.

Experiment 162.—The cortex cerebri, the cerebellum, medulla and spinal cord were examined, and all yielded the same results. With a magnification of 1200 diameters, the capillaries and vessels were found to contain chromatin bodies, exactly resembling Leishman bodies, except that they were smaller, measuring from 1 to 2 μ, much more frequently 1 μ, rarely as large as 2 μ. They were either circular or oval rings or had the appearance of the chromatin particles being situated at the two poles. Several drawings and photographs are given to illustrate their appearance and their numbers. Some of the capillaries show immense numbers, and in some transections of larger vessels, these bodies can be observed lying in a zooglœal mass.

Individual bodies exhibit some diversity in their form, indicating division. A large number of stained particles (which may be micro-nuclei) can be seen.

The Jinga trypanosome, as the accompanying drawing shows, is comparatively a large organism, as seen in the blood of a monkey, which was inoculated with it. Its oval macro-nucleus is much larger than these

chromatin bodies which are seen in the vessels. If these chromatin bodies, as Leishman would affirm, are the macro-nuclei of trypanosomes, then it is difficult to explain why a dozen or more of the chromatin bodies can sometimes be seen lying in a space which would be covered by one trypanosome. Still the trypanosomes may have degenerated elsewhere, and the macro-nuclei be carried into the capillaries. In view, however, of the researches of Captain Rogers regarding Leishman bodies being altered phases of trypanosomes, and the contention of Plimmer and Bradford *re* the existence of amœboid forms of trypanosomes, it is possible that these chromatin bodies may be some phases in the life of the trypanosome in the blood; and it may be mentioned in support of this (although I do not profess to dispute the opinion of biologists who have studied the question) that after a very careful search of a large number of sections, I have been unable to see a single trypanosome or a degenerated one, which is quite different to what one found in the brain of the rabbit inoculated with *Surra*.

Experiment 202. *Ox.*—This animal died within three months of infection, the same portions of brain were examined. It was only after some careful searching that I could find a few small vessels containing these chromatin bodies. A drawing is given to illustrate these vessels. It will be observed that there are a far larger number of minute, just visible, stained particles.

Addendum.—Since reading this preliminary note, by a new method of staining I have found trypanosomes, and what I believe are Plimmer and Bradford's amœboid forms in ox, Experiment 202.

Clearly, then, at any rate in the brain, the evidence of the existence of trypanosomes in the blood of animals dying of trypanosome disease may vary very considerably. In view of the fact that blood in which no trypanosomes can be detected microscopically, yet by culture experiments they may be obtained, it may be asked (although here, again, I do not pose as an authority) whether these bodies with chromatin particles can develop into trypanosomes.

In the vessels of the brain of the Ox 162, many leucocytes can be seen which have taken up the chromatin bodies. It may be mentioned that in these two cases there is no sign of meningo-encephalitis, and there was no diplo-streptococcal infection. The ganglion cells showed chromolytic changes, and there were *many minute capillary* hæmorrhages, probably due to plugging of the capillaries by the organisms.

Moreover, there were curious cells lying free in the vessels, which, however, I could not assert were not detached endothelial cells with nuclear changes, except that I have not observed such appearances before. (*Vide* drawing.)

Donkey inoculated with mule trypanosomes. In this case the central nervous system yielded no positive results.

Monkeys inoculated with different varieties of trypanosomes (including four certain cases of Sleeping Sickness), of which eight brains have been examined. The animals lived for varying periods, from a month or two to over one year. As the results were negative, I shall not give any particulars here.

The most obvious change found was the empty condition of the small blood vessels and chromolytic changes in the nerve cells. There was no peri-vascular cell-infiltration and no meningitis. The tissues of some of them showed diplococci.

In one case of Sleeping Sickness (Zurura Mya), a chronic case in which trypanosomes were found in the cerebro-spinal fluid during life without centrifuging, I was unable to find any perfect trypanosomes in sections of the central nervous system, but I found numbers of bodies which I thought might be altered forms, or fragments of degenerated trypanosomes and chromatin bodies, especially in the chronic inflammatory exudation of the subarachnoid space. Moreover, small capillaries could be found ruptured in the neighbourhood. This fact I have observed in other chronic cases, and suggests the possible mode of infection by trypanosomes of the cerebro-spinal fluid in the subarachnoid space.

I have had the opportunity, recently, of examining sections of a case of chronic basal meningitis with diplo-streptococcal infection. Sections showed in places a very marked peri-vascular infiltration of some of the vessels of the cortex, away from the primary source of infection, resembling, in some respects, some of the less chronic cases of Sleeping Sickness. I failed, however, to discover, amidst the cell exudution, those small round and oval bodies and fragments which I have found in the meningo-encephalitis of chronic Sleeping Sickness. But, in a case of basal meningitis occurring in a child, only the membranes about the base of the brain were affected, and no peri-vascular infiltration was found. There were numbers of small round and oval bodies, probably products of degenerated cells.

In my opinion, therefore, a series of culture experiments *in vitro* of different forms of trypanosomes, especially of "Sleeping Sickness" in cerebro-spinal fluid, would be of interest. This fluid, containing a mere trace of proteid, might lead to degeneration of these organisms, and products and fragments, similar to those found in the membranes, might be observed on microscopical examination of the centrifuged fluid. If no change in the trypanosomes occurred, infection of the cerebro-spinal fluid by diplo-cocci or diplo-streptococci might be undertaken.

Positive results by this method might help in deciding this difficult

point; whether the chronic inflammatory exudation is the result of the irritation caused by the trypanosomes or of their toxic products; and whether any of these small round and oval bodies, seen in great abundance in the chronic inflammatory products, are products of degenerated trypanosomes.

Addendum.—A full report of this investigation, with the abstract of the clinical notes of the cases, the photo-micrographs and drawings, will appear in the Reports of the Sleeping Sickness Commission.

By the kind permission of Major Leishman, I have since had the opportunity of examining a portion of the cortex cerebri of a monkey which died quite recently; this animal was inoculated with the blood of a case of Sleeping Sickness 18 months previously, and unlike any of the eight monkeys' brains which I have had the opportunity of examining, it shows a well-marked meningo-encephalitis. This very important fact was referred to by Major Leishman in the discussion that ensued, and a full report of the examination of the brain will be published by Captain Harvey.

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