ON THE POSSIBILITY OF THE OCCURRENCE OF TRYPANOSOMIASIS IN INDIA.

By MAJOR W. B. LEISHMAN, M.B., R.A.M.C.,
Professor of Pathology, Royal Army Medical College.
[From the Pathological Laboratory, R.A.M. College, Victoria Embankment.]

The recent discovery of trypanosomiasis in man by Dr. Dutton and Dr. Purdy, and the report of further cases by Dr. Murray, naturally lead one to question the possibility of the occurrence of this disease in other parts of the world than those originally reported—viz., the Congo and the Gambia. In the following remarks I hope to show that there is at least some ground for the belief that it may occur in India, and that this species of trypanosomiasis may be one of the indefinite varieties of fever occurring in that country, in which the presence of malarial parasites in the blood is not determined or is, at least, only incidentally noted.

The case upon which this theory is based belonged to such a class, whose general features I shall briefly describe before going into details with regard to the individual patient. For want of a better name I may speak of them as cases of "Dundump fever," because, as far as my experience goes, the patient usually came either from this cantonment or its immediate neighborhood. This station of Dundum lies about seven miles from Calcutta and is notoriously unhealthy, malarial fevers of all types, dysentery, and enteric being rife. It is excessively damp, and, in the rains, is practically a morass. Of the fact of its being said to be even a few feet below the level of the Hooghly, which flows within a mile or two of the cantonments. I had a short personal acquaintance with this station in 1890, but the present remarks refer to the features of this form of fever as presented by soldiers invalided on account of it from Dundum to the Royal Victoria Hospital, Netley, during the past three or four years. The cases were, as a rule, regarded as having been malarial in origin, and presented, on admission, an extremely degree of cachexia; it was, in fact, the severity of this cachetic condition and the frequency of its association with Dundum— and, more rarely, its immediate neighbours, Calcutta and Barrackpore—which gave rise to the idea that we were dealing with a specific type of fever, whose cause was unknown. Clinically, these cases presented no very definite features distinguishing them from other and commoner forms of tropical cachexia, the chief symptoms being an irregularly remittent type of fever, grave anaemia, progressive muscular atrophy, and great enlargement of the spleen, which now think to have been due to trypanosomiasis.

Blood regeneration was so marked, the count having risen within a few days from two to three millions, that it was deemed safe to resume the injections, and in larger doses of 30 c.c.m. Two such injections were given, one on March 10th and the other on the 11th. At once the blood count began to drop, the patient to become depressed, and on the night of the 11th, about 11 p.m., alarming symptoms suddenly developed, namely, choleic movements in the legs extending to the trunk, and a high fever and rapid feeble pulse. At 10.45 a.m. on the 12th severe convulsions of the muscles of the trunk set in, and the patient became dull and apathetic; at the same time the erythematicous condition of the skin became more pronounced and more dusky. The temperature was reduced by sponging, and by the 13th had fallen to normal in the morning, the general condition also undergoing marked improvement, and the blood count, which had again become seriously reduced, rising once more to 3,000,000. Although there had been some reduction in the number of trypanosomes in the finger blood it was not considered to be safe to resume the injections. When discharged on March 27th the patient had quite recovered from what, had it been pushed much further, might have proved a disastrous line of treatment.

Table of Temperature and Blood Observations during period of Horse-serum Injections.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
<th>Pulse</th>
<th>Amount of Horse-serum, c.c.m.</th>
<th>Red Blood Corpuscles</th>
<th>Leucocytes</th>
<th>Hemoglobin per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 26th</td>
<td>M.</td>
<td>101.0</td>
<td>10</td>
<td></td>
<td>3,000,000</td>
<td>2,500</td>
<td>9,500</td>
</tr>
<tr>
<td></td>
<td>E.</td>
<td>98.8</td>
<td>10</td>
<td></td>
<td>2,700,000</td>
<td>1,800</td>
<td>9,000</td>
</tr>
<tr>
<td>March 1st</td>
<td>M.</td>
<td>99.6</td>
<td>10</td>
<td></td>
<td>2,900,000</td>
<td>2,000</td>
<td>9,200</td>
</tr>
<tr>
<td></td>
<td>E.</td>
<td>98.6</td>
<td>10</td>
<td></td>
<td>2,700,000</td>
<td>1,800</td>
<td>9,000</td>
</tr>
<tr>
<td>and</td>
<td>M.</td>
<td>99.6</td>
<td>10</td>
<td></td>
<td>2,900,000</td>
<td>2,000</td>
<td>9,200</td>
</tr>
<tr>
<td></td>
<td>E.</td>
<td>98.6</td>
<td>10</td>
<td></td>
<td>2,700,000</td>
<td>1,800</td>
<td>9,000</td>
</tr>
<tr>
<td>and</td>
<td>M.</td>
<td>99.6</td>
<td>10</td>
<td></td>
<td>2,900,000</td>
<td>2,000</td>
<td>9,200</td>
</tr>
<tr>
<td></td>
<td>E.</td>
<td>98.6</td>
<td>10</td>
<td></td>
<td>2,700,000</td>
<td>1,800</td>
<td>9,000</td>
</tr>
</tbody>
</table>

Pure Drugs.—A Bill has, according to American Medicine, been introduced into the New York Legislature to prohibit the sale of defective, stale, or otherwise deficient drugs, and to punish persons who make substitution of another drug for the one called for by a customer. The Bill provides that drugs likely to deteriorate in time must be marked with the date of manufacture, and with a statement showing the quality, strength, and genuineness of the drug. A time limit must also be fixed after which the drug will be unfit for use. The Bill was introduced at the instance of the Medico-legal Society of New York.

REFERENCES


Pursh Drugs.—A Bill has, according to American Medicine, been introduced into the New York Legislature to prohibit the sale of defective, stale, or otherwise deficient drugs, and to punish persons who make substitution of another drug for the one called for by a customer. The Bill provides that drugs likely to deteriorate in time must be marked with the date of manufacture, and with a statement showing the quality, strength, and genuineness of the drug. A time limit must also be fixed after which the drug will be unfit for use. The Bill was introduced at the instance of the Medico-legal Society of New York.

On making smear preparations from the spleen pulp, it was struck by the curious appearance, among the spleen cells and
red corpuscles, of enormous numbers of small round or oval bodies, 20 to 30 in diameter, which corresponded to nothing I had previously seen with or had ever described. They stained faintly with methylene blue and with haematoxylin, showing with these stains a sharply contoured circular or oval shape, but no detailed structure; but, on staining them by Romanowsky's method, they were found to possess a quantity of chromatin, of a very definite and regular shape, which clearly differentiated them from blood plates or possible nuclear detritions. This chromatin appeared in the form of a more or less definitely circular mass or ring, applied to which, though apparently not in direct connexion with it, was a much smaller chromatin mass, usually in the form of a short rod set perpendicularly or at a tangent to the circumference of the larger mass. The outlines of the sphere or oval enclosing these masses of chromatin were only faintly visible by this method of staining. (See Fig. 1.) These little bodies were

their faculty of staining by the blue element of Romanowsky's stain. The macro-nucleus becomes denser and more circular in shape, and, together with the micro-nucleus, retains its chromatin reaction for as long as I have had the organs under observation. The vibratile membrane is rapidly shed, and, 12 hours after death, these may be seen in large numbers lying free among the dead parasites; occasionally they carry with them the micro-nucleus, attached perpendicularly or at an extremity, but more frequently, this is left behind in the spherulated body of the parasite. Forty-eight hours after death these vibratile membranes can no longer be detected, even by prolonged staining, and the outline of the body of the parasite can only be made out in a few cases. Further shrinkage of the body approximates the macro-nucleus to the micro-nucleus, if the latter has been left behind, and all that remains in the sharply-defined chromatin mass or ring of the altered macro-nucleus with, in many cases, the micro-nucleus closely applied to it, resembling in shape the young bud thrown out by a yeast cell. Fig. 2, which was taken from a lung smear 48 hours after death, shows several stages in this degenerative process.

As to their meaning I was at the time completely at a loss, nor could other observers, to whom I have from time to time shown them, give me any clue as to their nature. In two fatal cases of the same type of Dum-dum fever which occurred subsequently I failed to find anything of the same nature in the spleen, though in those cases this organ was not enlarged to the same extent as in the case of Private B.

It was only recently, while working with nagana, the trypanosoma of tsetse fly disease, discovered by Lieutenant-Colonel D. Bruce, R.A.M.C., that I came across the appearance in the blood and internal organs of a white rat, dead of this disease, which, I venture to think, present the key to the puzzle. On examining this animal forty-eight hours after its death, I found in the blood and organs bodies practically identical in shape and staining reaction with those I had met in the spleen of Private B. The same little circular masses of chromatin, mostly free in the blood, but, in the organs, some thickness, were seen, and, in many instances, a smaller chromatin dot or rod was noticed lying in apposition to the larger mass. As the blood of this rat on examination shortly before its death was found to be swarming with trypanosoma, there could be little doubt that these chromatin bodies represented the macro-nuclei and micro-nuclei of the adult organisms, and that these structures were practically all that remained of the parasites, the rest of the body and the vibratile membrane, being less resistant to degenerative change, having disappeared. Further experiments soon proved this to be the case, and it was easy to trace every step in these degenerative changes from the death of the trypanosoma onwards.

The white rat, as a rule, dies about ten days after inoculation, with enormous numbers of trypanosoma in the peripheral blood, in all stages of longitudinal fission. After death the parasites very rapidly lose their motility and also their typical flagellate form, shrinking up into ovoid or spherical bodies, which have, to a considerable extent, lost
the disease shall run the course of a true septicaemia, with numerous parasites occurring and multiplying in the general circulation, only when the experiment is performed on a susceptible animal. Certain of the internal organs, appears to be mainly the resistance of the particular species. The experiments of Bruce,6 Laveran and Mesnil,7 Plimmer and Bradford,8 and of Masini,9 have the widely separated limits of the time required by trypanosomes to cause death in hosts of different species investigated. Thus, the very susceptible rat dies in a few days, while the guinea-pig may live for several months. Speaking generally, the more resistant the species or individual, the less likely are we to find the organisms in the numbers harbored by the blood during life. Taking the white rat, again, as an example we find the parasites appearing in the blood three or four days after inoculation; then they increase enormously in numbers until, just before death, there may be almost as numerous as the red blood corpuscles. On the other hand, in the rabbit, which shows a considerably greater power of resistance, death being postponed frequently for a month or six weeks, parasites can only rarely be found, and frequently the most careful search, of moulage films fails to demonstrate a single trypanosome, the organisms in this case being found in large numbers either in some of the internal organs and the bone marrow, or forming accumulations in the lymphatics of certain regions, such as the eye or the genital organs.

It is thus evident that trypanosomiasis in an animal does not necessarily postulate the occurrence of the parasites in the peripheral blood, in numbers large enough to permit of microscopic detection.

Again, the chronic nature of the disease in the few recorded cases of trypanosomiasis in man, and the immunity which man appears to possess to infection by other varieties of trypanosoma, such as nagana, surra, dourine, mal de caderas, etc., would appear to show that man is very resistant to trypanosoma infection.

It is not, then, I think, unreasonable to assume, first, that when trypanosomiasis does occur in man the parasites are more likely to multiply and accumulate in the capillaries or lymphatics of the internal organs than to invade the general circulation, and that the tendency of the parasites to the internal organs during life of the host, and partly to the rapid degeneration and loss of characteristic shape which follow on the death of the organism and render its identification in blood films of sections of organs quite difficult. This difficulty is further increased by reason of the fact that only by Romanowsky staining can the macro- and micro-nuclei be rendered visible, and then only in smear preparations, since it is very hard to produce satisfactory chromosome staining in sections owing to the chemical alterations induced in the tissues by the various processes of hardening and embedding. I have tried to modify these processes in various ways, but so far without success.

With a view, then, to finding out whether some of these severe trypanosomiasis, or the cases of malignant fever, may not be due to trypanosomiasis, I would suggest that, as a routine practice, necropsies of such cases should include the staining of smear preparations from the spleen, lungs, and liver by Romanowsky’s, in the hope of finding some new degenerative forms of the parasites which I have described.

Increased facilities for the detection of trypanosomiasis during life are much to be desired. Positive results might be obtained by inoculation of a susceptible animal with blood from a suspected patient, and if the inoculated animal appears to have been successful in Dr. Manson’s case, and in the cases of Annett and Dutton, record that the pathogenicity of the trypanosoma in their case was not marked in white rats. Possibly the monkey might prove more susceptible to the trypanosomes than the guinea-pig or the white rat. If this be so, inoculation of the mixture after dissolving out the haemoglobin, as with those of Laveran and of Ross, may also give an increased chance of success, and in this connexion I may note that the modification of Romanowsky’s stain, described by me in the Barron Medical Journal,10 may be utilized for this purpose by mixing a little of the stain with twice its volume of water, pouring the mixture on to the unfixed film, and at the end of ten minutes, washing it off, when the film can be dried and mounted. It has also been suggested that larger quantities of blood may be examined by citrating and employing the centrifuge.

I would further suggest, in conclusion, that the procedure I have indicated might possibly be of service in the investigation of kala-azar and of sleeping sickness, both of which have recently been considered as perhaps due to trypanosomiasis.

REFERENCES.

THREE CASES OF TRYPANOSOMA IN MAN IN ENTEBBE, UGANDA.

By C. J. BAKER, L.R.C.P., M.R.C.S., Medical Officer, Entebbe, Uganda.

CASE I.—A native policeman came to the dispensary at Entebbe on March 12th, 1903, suffering from pain in the head and fever, his temperature being 102.5°F. I examined a fresh film of his blood for possible malarial parasites. Though unsuccessful in this direction, I discovered an elongated, actively motile body which, on closer inspection, I found to be a trypanosome. Dr. Moffatt, P.M.O. Uganda, and Dr. Castellani, of the Sleeping Sickness Commission, who both happened to be close at hand, and they were able to give their valuable confirmation to my diagnosis. I afterwards found about a dozen of these parasites in the admission, the patient, a native of the Uganda district, stated that he had never been outside the Uganda Protectorate, and had lived for the past 12 months in Entebbe, except when he had made three journeys to Masindi (Unyoro district). He had never to his knowledge been bitten by tsetse flies, or by leeches about a year previously. He had never had a similar attack before, and had been quite well up to two days before admission, when he began to complain of general headache. His temperature was 102.5°F., his pulse-rate 100 per minute, his respirations were normal, his organs on examination appeared perfectly healthy, the spleen not being enlarged, and there was no oedema of the skin. After admission his temperature on the second day reached 104°F., after which it began to fall, reaching normal on the third day. He underwent no treatment. At the same time the parasites gradually diminished in number and apparently disappeared from the blood on the same day as the temperature reached normal, and subsequent daily examination of his blood produced only a few parasites.

CASE II.—Kumour Saba, a native policeman, came to the dispensary on March 28th, 1903, suffering from headache and having a temperature of 101.5°F., I examined his blood and found a single trypanosome, which was also seen by Dr. Nabarro. On admission his temperature was 101.5°F., his pulse-rate 92, and there was the same slight general headache as in the first case. He stated he was one of the Lendu tribe who migrated from the west side of the Albert Nyanza about 1893, since when he had been in the British district of Uganda district, and he had never been bitten by any tsetse fly, but had been badly bitten by leeches about a year previously. He could give no history of having been bitten by any biting fly, and had had no previous similar attack, and was quite well up to the day before admission. On examination his temperature was normal on admission, and there was no oedema of the skin. Though I have since examined several films of his blood, I have not found a single trypanosome other than the one mentioned above. His temperature dropped to 99°F. on the second day, and became normal on the third and fourth day. All headache left him on the second day.

CASE III.—Jardien Murjan, a prisoner, was admitted to hospital on March 31st. On admission his temperature was normal, his pulse-rate 70 per minute, and he complained of severe headache. On examination of his blood I found several trypanosomes. His spleen was slightly enlarged but not tender, and he had slight oedema of the skin over the abdomen extending upwards over the lower ribs. He could give no history of a previous similar attack, and was