
By Surgeon-Major Ronald Ross, L.M.S.,

(With Note by Surgeon-Major Smyth, M.D., L.M.S.)

For the last two years I have been endeavouring to cultivate the parasite of malaria in the mosquito. The method adopted has been to feed mosquitoes, bred in bottles from the larva, on patients having crescents in the blood, and then to examine their tissues for parasites similar to the heamoméba in man. The study is a difficult one, so far, as there is no a priori indication of what the derived parasite will be like precisely, nor in what particular species of insect the experiment will be successful, while the investigation requires a thorough knowledge of the minute anatomy of the mosquito. Hitherto the species employed have been mostly brown and grey mosquitoes of the insect; but though I have been able to find no fewer than six new parasites of the mosquito, namely, a nematode, a fungus, a gregarine, a sarcosporidium (?), a coccidium (?), and certain swarm spores in the stomach, besides one or two doubtfully parasitic forms, I have not yet succeeded in tracing any parasite to the ingestion of malarial blood, nor in observing special protozoa in the evacuations due to such infection. Lately, however, on abandoning the brindled and grey mosquitoes and concentrating on a work on a new, brown species, of which I have not yet obtained more individuals, I succeeded in finding in two of them certain remarkable and suspicious cells containing pigment identical in appearance to that of the parasite of malaria. As these cells appear to me to be very worthy of attention, I have described them. These peculiar species of mosquito seems most unfortunately to be so rare in this place that it may be a long time before I can procure any more for farther study; I think it would be advisable to place on record a brief description both of the cells and of the mosquitoes.

The latter are a large brown species, biting well in the daytime, and incidentally found to be capable of harbouring the filaria sanguinis hominis. The back of the thorax and abdomen is a light fawn colour; the lower surface of the same, and the terminal portion of the body a light brown. The wings are light brown to white, and have four dark spots on the anterior nerver. The haustellum and tarsus are brindled dark and light brown. The eggs—at least, when not fully developed—are shaped curiously like ancient boats with rams at each end, and have black heads; taken from the concave border like banks of oars—so far as I have seen, a unique shape for mosquito’s eggs. The species appears to belong to a family distinct from the ordinary brindled and grey mosquitoes, but is an allied species here, only more slender, white, and much less voracious. My observations on the characteristics of these mosquitoes were not very careful, as when I first obtained them I did not anticipate any difficulty in procuring more.

On August 16th eight of them were fed on a patient whose blood contained four to refer crescents (and also filariae). Unfortunately four were killed at once for the study of flagellate bodies (flagellate cysts). Of the remainder two were examined on the 15th and 20th respectively, without anything being noted. The seventh insect was also killed on the 20th, four days after having been fed. On turning to the stomach with an oil-immersion lens I was struck at once by the appearance of some cells that were slightly nodular, and that the cells of the mosquito’s stomach usually are. There were a dozen of them lying among (or within?) the cells of the upper half of the organ, and, though somewhat more solid than these, still very delicate and coloured the eye at once; and as they were 16 in diameter when not compressed (that is, considerably larger than the largest heamoméba in man); the outline sharp but very fine; the contents full of stationary vacuoles; and no sign of apparent nucleus, contractile vesicle or ameboid or incellular movement. So far it would have been impossible for any person to be very familiar with the insect’s anatomy to have distinguished them from the neighbouring cells; but the now arrested attention was the fact that each of these bodies contained a few granules of black pigment absolutely distinct in appearance with the well-known and characteristic pigment of the parasite of malaria (large quadrants and crescent-derived spheres).

The granules were more scanty in comparison to the size of the cells than in the heamoméba, numbering from 10 to 20 in each. They were not dispersed throughout the cells, but were collected in groups, or arranged in lines transversely or perpendicularly, or in a small circle round the centre (just as in some forms of the heamoméba). They were black or dark brown, refractive on the eye, and in some cases, on examination, they showed rapid oscillation within a small range, but did not change their position. Owing to their blackness, so different from the bluish, yellow, and green granules of the heamoméba, they were easily detected, and I was not surprised that I have not confounded them with normal objects. In short (except perhaps that rods were shorter or absent) these granules of pigment were indistinguishable from those of the heamoméba.

The eighth and last mosquito was killed next day, five days after having been fed. The stomach contained precisely the same cells, 21 in all, again toward the cesophageal end of the organ. In this case, however, they were distinctly larger and more substantial than in the seventh mosquito, and had a decidedly thicker outline. The size (along the major axis) appeared now sometimes to reach nearly 20 μ, on a rough computation made without a micrometer. There thus appeared to be a marked increase in bulk and definition between these eighth and fifth days, suggesting that they had grown in the interval.

Both specimens were irrigated with 40 per cent. formalin, and sealed. The result of the formalin was, as anticipated, that the bodies became slightly more visible than before, as compared with the stomach of the previous dissections of mosquitos (I suppose I must have examined quite a thousand
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In quarter fever, and in Albutt's System of Medicine (1897) the diagrams of the quartan parasite give a very good general idea of his appearance in the specimen I have attempted to describe, the only difference being that the former seem to contain a larger number of granules.

Preparation II.—This is a specimen of a mosquito dissected on the fourth day after feeding on a forearm containing crescents, and mounted in the same way as Preparation I. The only difference observable between this preparation and the other is that the pigmented cells are on the whole slightly smaller, ranging between 0.0175 mm. and 0.0075 mm., and are more hyaline and altogether more delicate in structure.

Mr. Bland Sutton writes: "I have examined Surgeon-Major Ross's specimens, and had an opportunity of discussing the matter with Dr. Manson, and thoroughly endorse his opinion. We must wait until experiments demonstrate the subsequent fate of the intracellular parasites before coming to a positive decision. It is a particularly interesting and important research, and one which the skill and enthusiasm of Surgeon-Major Ross will doubtless bring to a satisfactory conclusion."
black granules which they contain are pigment derived from the parasite of malaria. (1) The pigment: The pigment is found in the form of very minute needles, and in small spheres of varying sizes, but none of them larger. This coincides exactly with the forms of pigment observed in the human subject when patients have died of malaria. In the cells of the spleen of patients who have died at Sierra Leone, I found in some of the other blood cells, the pigment, in others both needle-shaped and minute spheres in the same cell, in others only spheres of pigment. By the time the cells have reached the liver, the pigment in my cases had all become spherical. Even if I had not known that I was looking at a malarial pigment, the fact that I have been fed on blood containing crescents, the appearances would at once have suggested to me that I was looking at malarial pigment. The position of the pigment in the cells is accurately described by Surgeon-Major Ross. In some of the cells it is irregularly distributed in the periphery, and in others in a small circle towards the centre. There are also small pigment spheres scattered throughout the preparations, without any apparent connection with cells, and the pigment spheres when it was apparently extra-cellar, were somewhat larger than those which were within cells. (2) The cells: The cells have the outlines of epithelial cells, and they stand out clearly against the other structures in the preparations. I believe they are the ordinary epithelial cells of the stomach of the mosquito which have undergone for the purpose of feeding. I saw the preparations twice. The first time I saw them these cell-like bodies stood out very clearly in a field in which the other elements were very transparent and indiscernible. The whole field was filled with these extraordinary refractive appearances which seemed to be both outside and inside the cells. Before I examined the preparations the second time some change had taken place (probably by the introduction of cedar oil under the edge) which caused the tissues to lose the light and clarity which they enjoyed when they were in the mosquito's stomach, and it became evident to me that when one of the epithelial cells of the stomach is infected with malarial pigment it undergoes changes—the body is altered by the presence of the pigment, and make it stand out clearly on a transparent background. The small circles of pigment which are to be seen in some of these bodies are so arranged as to render it almost certain that they are arranged round the nucleus of the epithelial cell. I found in the Sierra Leone cases that the nuclei of the large white cells of the spleen that were not much infected by pigment, that the pigment was first found in the outer part as fine needles, and afterwards was grouped in spheres round the nucleus. How can we account for the cells standing out so much? When the cells are stained with Romanowsky's stain? I consider this to be analogous to what takes place in the endothelium of the blood vessels in the capillaries of the brain, spleen, and liver in the Sierra Leone cases. The endothelial cells of the capillaries which contain pigment, of many of them do contain pigment altered in their substance, shown by their taking up an eosin or cochineal stain when the healthy cells that do not contain pigment do not stain. In the human subject these infected endothelial cells are black or dark brown in colour. This was described in the paper which I read at the Royal Medical and Chirurgical Society on October 25th, and illustrated by microscopic preparations. Reasoning from analogy, I infer that the epithelial cells in the mosquito's stomach that absorb pigment are injured by the pigment, are altered by it, and would eventually be killed by it, and that the injured or dead cells possess a refractive property which is not present in living and uninjured cell, and stands out clearly in an otherwise almost transparent field. It is quite natural to expect that if a mosquito is fed on blood containing crescents, the pigment of these crescents should be found in the stomach of the insect, and as animal cells possesses the property of taking up very minute granules (the liver cells as well as the capillary endothelium take up pigment), it is reasonable to infer that they might be found in the cells of the mosquito. I have seen these crescents, and in accordance with the views of the late Surgeon-Major Ross, I have no doubt that they are parasites, and I think, in view of the matter, and requires further explanation, is why this appearance is found only in one species of mosquito. I see no trace of the parasite of malaria itself in these preparations, but, as they are not stained, it does not necessarily follow that it is not there. It will be evident, from what I have remarked, that I consider that these appearances do not represent parasites, and that the extra-corpooreal stage of the parasite of malaria, which Koch and Pfeiffer were, I believe, the first to suggest, might be found in the cells of the mosquito. Ross's investigations. His published papers, and not the least the one to which this note is appended, show that he is a patient, skilful, and indefatigable observer. He has already added considerably to our knowledge of the mosquito and its parasites, and after what has been ascertained of the association of the mosquito with the filaria sanguinis hominis (in connection with which Dr. Manson's name is so distinguished), no one can believe that any information regarding the parasites of the mosquito may not turn out to be of great practical value, as it certainly is of considerable scientific interest. It is therefore, I consider, very important that Surgeon-Major Ross, with his exceptional capacity and opportunities, should continue his investigations not only regarding the mosquito in connection with the parasite of malaria, but also by working in any other line which his observations point out to him as possibly leading to a solution of the unsolved riddle of the existence of the parasite outside the human body, for outside the human body we know it does exist. It may be that information which will be of interest in the welfare of India, and indeed in that of the whole tropical world, that men like Surgeon-Major Ross should be found willing to devote themselves to such an arduous and fatiguing work. Only men endowed with unusual enthusiasm could be found to devote themselves anew to what has done to such a task. In the interests of science it is therefore most desirable that support, assistance if required, and every facility should be given to him to enable him to continue his researches."

A LIMITED outbreak of diphtheria occurred recently in Leeds, which at first seemed to be associated with the milk supply. Further inquiry, however, proved that the patients had only been getting milk since their illness had commenced. The infection was probably spread by school influences.

ABERDEEN ROYAL INFIRMARY.—In the old Aberdeen Royal Infirmary two of the wards were named after the Queen and Prince Albert respectively, and by permission of the Queen two of the wards in the new medical pavilion will be similarly designated. Wards have also been named after Princess Beatrice and Princess Louise.

THE LIVERPOOL MEDICAL INSTITUTION.—Under the will of the late James Motter Turnbull, M.D., F.R.C.P., whose death was announced in the British Medical Journal some weeks ago, the Liverpool Medical Institution receives a legacy of £500 free of duty. Dr. Turnbull was President of the Institution for the years 1876-77.