PART V.—DISTRIBUTION OF THE LEISHMAN BODIES IN THE TISSUES.

The following tissues in this case were examined, smears on slides as well as stained sections being studied in most cases: liver, spleen, intestine, mesenteric vessels, mesenteric glands, pancreas, kidneys, suprarenal capsules, brain, pia mater, choroid plexus, skin (small ulcers and petechial patches), and the femoral lymphatic glands draining the area of infected skin; bone-marrow, lung, testis.

The following methods for preparing the tissues for section cutting were employed:

Small pieces of tissue were placed in 80 per cent. alcohol for twelve hours, then in 60 per cent., and finally in absolute alcohol for similar periods. The hardened tissues were now dropped, first into a mixture of equal parts of xylol and alcohol, and kept there twelve hours, then in pure xylol for twelve hours. This was followed by twenty-four hours in a paraffin bath. The tissues were now embedded and cut into sections 3 to 5 μ thick. The methods of staining used were:

1. Leishman's (chromatin staining in sections).
2. Hæmatoxylin and eosin.
3. Hæmatoxylin and Van Gieson (5 cc. of a 1 per cent. solution of rubin in a saturated solution of picric acid).
4. Löffler's methylene blue and eosin.

Of these methods that of Leishman gave the best results, as the parasites in the tissues were more clearly defined than by any other. It is a somewhat long method (three hours) and requires close supervision, but it is most satisfactory when there is time to carry it out. The quickest good method is that obtained by using hæmatoxylin with Van Gieson's solution as a counter-stain. An alcoholic solution of hæmatoxylin, rendered more active by keeping, is placed on the prepared section for ten to fifteen minutes. The red-brown stained section is now washed in water till it is of a blue colour.

Safranin was also tried later, but did not give very satisfactory results.
(about a minute's washing), it is then dipped into a bottle containing Van Gieson's counter-stain for ten seconds, rewashed, dehydrated, cleared and mounted. By this method the Leishman bodies and cell nuclei are stained a brownish-red; fibrillated connective tissue fibres and nerve tissue, such as neuroglia, ganglion cells, &c., are stained pink, and endothelial cells and connective tissue yellow. Thus this is a very suitable stain for mixed tissues (intestine, skin) or nerve tissues.

Eosin as a counter-stain did not give such good results as Van Gieson's solution. The method of staining by prolonged immersion (twenty-four hours) in Löffler's methylene blue followed by eosin as a counter-stain did not give satisfactory results.

I am greatly indebted to Lieutenant Proctor, I.M.S., and Lieutenant Hyde Hills, R.A.M.C., for help in this part of the work. Lieutenant Proctor helped me to prepare tissues, cut and stain sections in the earlier part of the investigation, while Lieutenant Hyde Hills has done nearly all the section cutting and much of the staining during the last two or three months. It was also at his suggestion that Van Gieson's counter-stain was employed.

Over a hundred sections and many films were examined, including some kindly sent me by Lieutenant Proctor.

The Liver.—Under low powers of the microscope the most marked change was that of increase of the connective tissue in the portal areas, and also in between the columns of liver cells in the lobules. It was manifest that a considerable degree of inter- and intralobular cirrhosis was present, the columns of liver cells being broken up by bands of connective tissue, while around the portal vessels there was much young connective tissue and the liver capsule was decidedly thickened. This marked cirrhotic condition of the liver was not found in the case of R——, reported in the Journal of March, 1905, when the parasitic infection of the liver was more intense than in this case, and from the general macroscopic and microscopic appearances of the liver in this case. I am convinced that the cirrhosis was largely independent of the effects produced by the parasite; however, a very slight degree of liver interlobular cirrhosis was found in R——'s case also, and it is quite possible that the constant invasion of the portal areas of the liver by the parasite may set up some degree of cirrhotic change.

Under the one-sixth inch lens and four ocular the cirrhotic change was confirmed, and the liver cells lying in the portal zones, especially of the liver lobules, were found to be misshapen by the presence of the new-formed fibrous tissues. Several of the liver
cells were atrophied, many contained fat globules. Leishman bodies looking like small cocci under this magnification were seen lying in cells, with light-staining cytoplasm between the liver cells. The parasitic infection appeared to be more intense in the portal than in the hepatic zones of the lobules.

Under the one-twelfth inch lens the fact that the parasites were enclosed in cells or lying in the capillaries was confirmed. No Leishman bodies were seen in the liver cells themselves. The cells which contained the parasites could be classed as follows:

(a) Practically unaltered endothelial cells; these contain generally only one or two parasites.

(b) In cells varying in size from 15 to 40 µ of various shapes; long, narrow, oval, round, or quite irregular; generally mononuclear, but sometimes containing two nuclei. The nuclei (8 to 14 µ) varied in shape as much as the cells themselves and were long oval, round, kidney-shaped, or irregular; many of these nuclei were much fragmented. These cells, both cytoplasm and nuclei, have similar staining reactions to the unaltered endothelial cells, above described (a), with the exception of cells, which contained great numbers of bodies; these had a more granular and darker staining cytoplasm than unaltered endothelial cells, an appearance possibly occasioned through changes in the cell cytoplasm by parasitic inclusion. These cells were either attached by one side or by a cytoplasmic process to the capillary wall, or lay loose in its lumen.

(c) In masses of cytoplasm without nuclei.

(d) In large mononuclear and in polynuclear white blood cells.

Prolonged study of many sections tends to show that these parasite-infected cells are endothelial in nature, for every gradation in size or shape, from the unaltered typical endothelial cell containing one or two bodies (its cytoplasm as yet unaffected by the parasitic invasion) to the large, round, oval, or irregular cells packed with Leishman bodies, was seen. Many of the cells were vacuolated as if the contained bodies had fallen out, the cytoplasm in some was reduced to a narrow rim surrounding a cavity filled with the parasites. Some of the cells, besides containing Leishman bodies, occasionally also contained red blood cells and rarely leucocytes within their cytoplasm. The evidently phagocytic nature of these cells did not affect the conclusion as to their endothelial nature, as the phagocytic character of vascular endothelium is well known.

A source of considerable difficulty in determining the presence
of Leishman bodies in cells is occasioned by the presence of the marked nuclear degeneration (alluded to above) in many of the large cells, many of the masses of chromatin from the degenerating and breaking-up nuclei closely resemble the dot of the Leishman body and occasionally a thin fragment like a rod might be lying close by, rendering it sometimes quite impossible to be certain whether the object in the cell protoplasm was a parasite or a chromatin granule (this condition is shown in many of the illustrations). Owing to this difficulty a rule was made never to consider a doubtful object a parasite unless there was a distinct rod and dot and no chromatin granules were scattered about in the cytoplasm of the cell examined. Besides the large cells referred to, all the other blood elements were met with in the liver capillaries, viz., red blood cells and leucocytes of various kinds. Leishman bodies were found in a few instances in large mononuclear blood cells and rarely in polymorphonuclear leucocytes; Leishman bodies were only very occasionally seen in the endothelial cells lining the portal or hepatic interlobular veins. They were also occasionally found in large mononuclear white blood cells in these vessels. The hepatic artery (interlobular) was not infected, though bodies were seen in one instance in the lymphatic vessels around an artery. No undoubted bodies were seen in the liver cells.

Examination of smears taken post mortem from the liver proved that both the blood elements and parasites seen in the large cells were included in the cells and not superimposed.

The mesenteric gland—under a low power—the lymph sinuses and lymphatic spaces, appeared to be more dilated than in a healthy gland and the lymphoid cords smaller, in fact, fewer lymphoid cells were present than one generally sees in a section from a healthy lymphatic gland. Under the higher power (see fig. 1, Plate II.) this gland was found to be very richly infected with Leishman bodies. The parasites were found in cells which closely resembled, both in staining reaction and position, the parasite-holding cells of the liver. They were situated in the lymph sinuses or in the lymphatic spaces. In the former situation they either lined the vessel wall or lay loose in it. In the lymphatic spaces these cells appeared to line the space, a mass of the cell cytoplasm filled with parasites bulging into the space. Occasionally the parasite holding cells were very large (40 μ), one of which would then fill a small reticular space. More usually three or four such cells lined or completely filled the spaces. A lymph sinus of the gland will be seen in fig. 1, Plate II., to contain parasitic infected cells which line
PLATE I.

Fig. 1.—Represents a section of the liver. The parasites will be seen to infect lightly staining cells lying in the capillaries between the columns of liver cells of the hepatic lobules. Two or three of these lightly staining cells are obviously practically unaltered endothelial cells lining the capillary, while the larger irregular-shaped cells are of similar nature, but altered in appearance, owing to the number of parasites they hold. One of these cells also contains a red blood cell enclosed in its cytoplasm.

Fig. 2.—Illustrates a lymph sinus in the enlarged femoral lymphatic gland. The trabeculae are much thickened owing to the marked fibrous hyperplasia present in this gland. The parasite-holding cells are similar in nature to those shown in the liver section, viz., endothelial.

Fig. 3.—Section of bone-marrow, showing the reticular spaces lined by endothelial cells, many of which contain parasites, while one such cell also contains a lymphocyte and a large mononuclear myelocyte or blood cell. The spaces are filled with myelocytes of various kinds—hyaline mononuclears, fine and coarse eosinophile cells, and nucleated red blood cells. One hyaline mononuclear myelocyte contains a parasite. Several parasites are seen in two cells (forming two sides of an incompleted space). These cells appeared by their shape and position to be possibly reticular and not endothelial cells.
PLATE I.

To illustrate paper by Captain J. C. B. Statham, R.A.M.C., "A Case of Kala Azar."

These sketches were made from sections as seen under a one-twelfth inch lens, and drawn with a camera lucida eye-piece. Magnification about 400 diameters.
its walls or lie free in its lumen, and some of the smaller reticular
lymph spaces are lined or filled by similar cells. Besides these
endothelial cells the gland sinuses and spaces contained blood
elements, principally lymphocytes and large mononuclear blood
cells; occasionally these cells were contained within the endothelial
cells.

Parasites were also found in the connective tissue cells which
help to form the walls of these spaces. These cells may be looked
upon as transitional endothelial cells, for the ultimate endothelial
cells of capillaries and lymphatics get more and more widely sepa­
rated from each other, and then join by their processes with the
connective tissue cells forming the spaces. No hard-and-fast line
can be drawn between these recticular cells and the endothelial
cells which join on to them. This condition is well seen when
the ultimate endothelial cells of a capillary are seen to be merging
into and joining the connective tissue cells of the lymph spaces.
Leishman bodies were also found in a few large mononuclear white
blood cells lying in the pulp.

Study of film smears confirmed what had been seen in the
sections.

The Mesenteric Vessels.—No marked change and no parasites
could be found in a section obtained from a mass of these vessels
bunched together and hardened. For some reason, however, the
sections from this time were a failure; the block has unfortunately
been misplaced, and consequently no definite conclusions can be
come to as to the presence or absence of parasites in this situation.

The Intestine (section from the large intestine through a
petechial patch).—Under a low power very few pathological
changes could be seen in the intestine. The glandular layer of
the mucous membrane was broken down in places, but this
was probably largely accidental. The capillaries of the submucosa
were somewhat dilated, and here and there there was some cell
extravasation from the capillaries. The lymph spaces between
some of the crypts of Lieberkühn showed similar cell infiltration.
There was much blood pigment scattered about in the submucous
tissue. Under the one-twelfth inch lens Leishman bodies were
found to be very few and far between; several sections were
searched before their presence was assured.

The bodies were found in three situations:

(1) In what appeared to be an endothelial cell of a lymphatic
vessel or in a connective tissue cell near it, in the tissue between
two crypts of Lieberkühn.
(2) In the endothelial cells lining a capillary.

(3) In two connective tissue or white blood cells lying among several which had been extravasated from a capillary.

The cells in which the bodies were lying had all the characters, both by position and staining reaction, of either endothelial or connective tissue cells. No bodies were seen in any of the gland cells. A body was found in a large mononuclear cell, lying in a capillary. The position of the bodies in the intestinal tissue is shown in fig. 2, Plate II.

**Femoral Lymphatic Gland** (see fig. 2, Plate I.).—The section examined was derived from one of the enlarged femoral glands, which drained the area of skin containing ulcers and petechial patches. Under a low power a marked degree of fibrous hyperplasia was found present, thick bands of fibrous tissue intersecting the gland in all directions. The lymph spaces were often much compressed by these bands.

Under the higher power Leishman bodies were found in similar cells to those seen in the mesenteric glands, viz., endothelial cells of the lymph sinuses, and in the cells lining the lymph spaces; occasionally also in the connective tissue cells already described as intimately associated with them. The parasitic infection was not nearly so intense as in the mesenteric gland.

**The skin sections** were taken from one of the petechial spots and from one minute ulcer. Under the low power the skin, like the intestine, did not show marked pathological changes. The capillaries were dilated, and very occasionally surrounded by extravasated white blood cells. There was no evidence of ulceration, evidently the minute ulcer had been missed in section, but there was slight infiltration of the subcutaneous layer, and the layers of the corium itself were in one place separated and divided by a space filled by blood débris. Under the high power the most marked change present was the infiltration of the skin and subcutaneous tissue with blood pigment. These facts increased the difficulties of a search for Leishman bodies already rendered trying by the constant presence of nuclear degeneration and fragmentation in the cells. After careful search, however, a very few undoubted parasites were seen; they were situated in similar positions to those found in the intestine, viz., in capillary endothelial cells, and in two or three cells, probably white blood cells or young connective tissue cells, extravasated from a capillary.

It may be noted that the distribution of the parasites in the intestine and skin presented many analogies. In both skin and
intestine the macroscopic changes were diffuse but slight, in both the parasites sparsely distributed.

It is possible, of course, that the portions of skin and intestine examined were not those most richly infected, the slight nature of the macroscopic changes in both cases rendered selection difficult. In both cases, as was reasonable to expect, the lymphatic glands draining the infected areas were more richly infected than the portions of tissues situated in those areas. This diffuse skin and intestinal distribution looked as if the parasites had come to these situations from the general circulation. If this was so, the slightly richer parasite infection of the portal over the hepatic areas seen in the liver lobules would be accounted for, for these portal areas would have three possible sources of parasitic supply: (1) From the general circulation through the hepatic artery; (2) and (3) from the portal vessels and lymphatics coming from an area of intestine possibly already infected with parasites from the general circulation. This possibility should, I think, be borne in mind in order to avoid any hasty conclusion, that because the portal areas of the liver lobules were more richly infected than the hepatic, therefore the primary parasitic infection must come from the intestine.

The Spleen.—Under the low power (two-thirds of an inch) of the microscope the following changes were noticed:—

(1) The spleen capsule was slightly thickened.

(2) The capillaries of the organ were much dilated and engorged with blood.

(3) The lymphoid elements seemed reduced in amount, and the Malpighian corpuscles looked smaller than normal.

Under the one-sixth of an inch lens the spleen was found to be richly infected with parasites, the intensity of infection varied, it was patchy. Under the one-twelfth of an inch lens the bodies were found in cells of a type similar to those described when speaking of the mesenteric gland sections, viz., the endothelial cells of capillaries and reticular lymph spaces, more rarely in reticular cell themselves. Fig. 1, Plate III., shows this condition and also illustrates another point, viz., that the endothelial cells of large vessels are scarcely ever infected with parasites, whereas where the blood-vessels break up into their ultimate capillaries and these merge into the reticular spleen, there we find the endothelial cells infected. It looks as though the parasites, or rather the white blood cells which carry them, cannot be taken up by the phagocytic endothelium till the circulation is much slowed down.

Besides these cells, Leishman bodies are also found in large
PLATE II.

Fig. 1.—Represents a section of the mesenteric gland seen under the one-twelfth inch lens. A lymph sinus is seen merging into the reticular lymph spaces of the gland. The walls of both the sinus and these spaces are lined in many places by endothelial cells, several of which contain parasites, and in two or three instances white blood cells also. One of these phagocytic cells (a large mononuclear blood cell) will be seen to be infected by a parasite. This is probably the manner in which the endothelial cells become primarily infected, though secondary cell-to-cell infection is largely influential in spreading the parasitic infection once it has commenced in an organ.

Fig. 2.—Shows two capillaries from the submucous tissue of the large intestine (cecum). In the capillary seen in longitudinal section an endothelial cell is infected, while several parasites will be seen among the cells extravasated from the other capillary (transverse section).

Fig. 3.—Section of suprarenal body. Shows a space, possibly a lymph space, near the capsule to contain an endothelial cell infected with parasites.
To illustrate paper by Captain J. C. B. Statham, R.A.M.C., "A Case of Kala Azar."
(12 to 18 \(\mu\)) mononuclear "spleen" cells; these cells stain a more decided blue than the endothelial cells, and have a more definite round outline. The nuclei of these cells are usually round (8 to 12 \(\mu\)).

Parasites were also met with in large mononuclear blood cells. Besides Leishman bodies, the phagocytic endothelial cells sometimes also contain red and white blood cells, or vacuoles may be seen in the cell cytoplasm of such sizes and shapes as to suggest that these blood cells have been present at one time and have fallen out (probably in preparing the sections and smears). Parasites were not found in the Malpighian follicles or in the lymph cords, never, in fact, when there was any aggregation of lymphoid tissue; it was, however, difficult to make out the condition of the endothelial cells in these areas, owing to the large numbers of lymphocytes present.

**Bone-marrow.**—The paraffin blocks of bone-marrow from this case unfortunately got lost, but several smears on slides had been preserved and were examined. These marrow films showed the parasitic infection to be more intense in the bone-marrow of W—'s than in R—'s case (JOURNAL ROYAL ARMY MEDICAL CORPS, March, 1905). As the distribution of the parasites in these films in the two cases appeared identical, sections of bone-marrow from R—'s case were examined (see fig. 3, Plate I).

Under the low powers of the microscope the intertrabecular marrow spaces appeared larger than normal, but this may have been due to the spaces being in many places emptied of myelocytes in preparing the sections.

Under the one-twelfth inch lens the parasites were chiefly found in the large lightly staining cells, already referred to as phagocytic endothelial cells. These cells were situated in the intertrabecular marrow spaces, lining them and often bulging into, or even nearly filling them. These cells were often very large indeed (50 \(\mu\)), contained one, sometimes two, nuclei, and occasionally included red and white blood cells as well as parasites in their cytoplasm (see fig. 3, Plate I.). It was difficult to decide whether some large cells which had several nuclei-like bodies were megalocytes, or were endothelial cells containing lymphocytes, but undoubted megalocytes were also seen in the sections.

Leishman bodies were also seen in:

1. Large neutrophile myelocytes (14 to 18\(\frac{\mu}{\mu}\)) with round or kidney-shaped neuclei (Cornil's myelocytes).

---

1 These measurements were made in film preparations and would therefore be slightly greater than if taken from the shrunken cells of alcohol-hardened tissues seen in the sections.
PLATE III.

**FIG. 1.—**Section of spleen. Shows a capillary merging into the lymph spaces of the spleen. Many endothelial cells contain Leishman bodies, and these parasites are also seen in cells which are possibly reticular cells. Some of the endothelial cells have also taken up white blood cells.

**FIG. 2.—**Section of lung, slightly reduced in size, from the drawing taken under the one-twelfth inch lens. One complete alveolar space is represented, while portions of five others are also illustrated. Leishman bodies may be seen in two places: (1) in what appears to be endothelial cells lining an interalveolar capillary in transverse section; (2) in the wall of an alveolus. The cell which contains them may be either lining the alveolar wall or the capillary which runs in it. The endothelial cells of capillaries and alveoli are here in such intimate association that it is impossible to differentiate them.
To illustrate paper by Captain J. C. B. Statham, R.A.M.C., "A Case of Kala Azar."
(2) Smaller neutrophile myelocytes (Ehrlich's).
(3) Large (12 to 18 \( \mu \)) round myelocytes with hyaline blue staining protoplasm and containing one, sometimes two, nuclei.
(4) What appeared to be large mononuclear blood cells.
Parasites were not found in the coarsely eosinophile myelocytes and nucleated red blood cells present in the marrow sections.

The Lung.—The portion of lung examined was taken from one of the congested bases. Under the low power the capillary spaces of the sections examined were dilated and full of blood corpuscles. There was no extravasation of white blood cells into the alveolar spaces, and no marked proliferation of the alveolar endothelium.
Parasites were found to be very scarce in W—'s lung sections. They were seen in :
(1) The endothelial cells of an interalveolar capillary. This vessel is seen in cross section in fig. 2, Plate III., and lies in a mass of three intersecting alveolar walls.
(2) In a cell lining an alveolar space, or the endothelial cell of the capillary in the alveolar wall; the intimate nature of the connection between alveolar and capillary endothelium makes it almost impossible to decide which.
(3) In a very few large mononuclear and polymorphonuclear white blood cells lying in the alveoli.

Suprarenal Capsule.—Section taken near cortex. Under low power of the microscope no pathological changes in the gland were remarked. Under the one-twelfth inch lens Leishman bodies were only found in one place—in a lightly staining cell lining a space just below the capsule, this space was probably a lymph sinus in cross section (see fig. 3, Plate II.).

Pancreas.—Smears only examined. No bodies found. (No parasites were found in sections from R—'s pancreas either.)

Kidneys.—Only two bodies were seen in the kidney section, they lay in the glomerular tufts, enclosed in what must have been endothelial cells.

Brain.—No parasites found in the sections from the cerebral cortex examined.

Pia Mater.—No Leishman bodies were seen in a small petechial spot in the pia, when this membrane was stretched on a slide, dried and stained.

Choroid Plexus.—The sections were not good ones, but two undoubted bodies were found in the endothelial cells of a capillary.

When this investigation had been practically completed, I read Marchand and Ledingham’s account of the distribution of the
Leishman body in the tissues of a case they examined. I had already come to the conclusion that the cells in which the parasite is most frequently found were endothelial, in nature, or were reticular cells—as Christophers had found before me. Marchand and Ledingham, while acknowledging that many of the parasite-holding cells in the liver were endothelial in origin, yet considered that the majority were special phagocytic cells derived from the spleen. After reading their paper, I still see no reason to alter the opinion I had come to that all these cells were really endothelial, much changed in shape in many cases by the parasitic invasion. Every gradation of parasite-holding cell between the practically unaltered endothelial cell containing one or two bodies, to the giant irregular-shaped cells filled with them, were met with in my slides. This swollen, altered appearance of the endothelial cell of the liver, spleen, bone-marrow, and lymphatic glands is met with in many diseases besides kala azar, such as toxæmic affections and in hyperplasias of these organs. The fact that these endothelial cells should be so phagocytic in character, englobing the parasites in such numbers and occasionally taking up white and red blood cells, is not surprising, considering their known phagocytic power. In malaria these cells often contain malarial pigment, while the researches of Gilbert, Carnot, Leon, Werigo, Lemaire and others have shown that they similarly take up bacteria and also pigments when these latter are injected into animals. Domini and Larier (quoted by Gilbert) have shown that the well-known blood-forming function of the liver present in the foetus is occasionally revived in persons suffering from toxæmic infections, and this fact might help to account for the presence of the red blood cells in some of these endothelial cells of the liver capillaries.

The parasites appear to have a wide distribution in the tissues, though the intensity of parasitic infection varies very strikingly. In R—'s case, as well as the present one, the liver, spleen and bone-marrow were much infected. In W—'s case the mesenteric and lymphatic femoral glands were also full of parasites, while in both cases the lung, kidney and suprarenal gland, though infected, contained extremely few bodies. The parasitic infection of the intestine and skin in this case was slight (altogether absent in that of R—). This variance in intensity of parasitic infection is due, I think, to the fact that the large mononuclear (and occasionally polymorphonuclear) white blood cells are more liable to be lodged and taken up by the phagocytic endothelial cells in such situations as the spleen, bone-marrow, lymphatic glands and liver, and these
white blood cells are probably the means by which the parasites are carried from one part of the body to another (I have never found the parasites in the peripheral circulation). Once the infection of an organ has taken place, however, the further spread of the infection in that organ is probably also caused by cell-to-cell infection; the parasites, once in the endothelial cell, rapidly multiply—the over-crowded degenerated cell ruptures—and if this occurs, say in the lymph spaces of the spleen, the parasites are rapidly taken up by an adjoining endothelial cell. It is only by the rapid intracellular multiplication of the parasites in the cell, and by the possibility of cell-to-cell infection, that we can account for the intense infection of some of the spleen and lymphatic gland areas—I have counted as many as 300 bodies in four endothelial cells lining a lymph space in W—'s mesenteric gland.

**General Remarks and Conclusions.**

(1) The disease in W—'s case was probably contracted in Calcutta, for he developed symptoms of kala azar almost immediately after arriving in Poona from Calcutta, and a disease like kala azar has almost certainly a considerable incubation period.

(2) It will often be difficult to find the Leishman body in kala azar in the absence of splenic puncture. The results of blood, sputum, urine and faecal examinations shown in this case illustrate this.

No alternative satisfactory method to that of splenic puncture has yet been devised. In repeated attempts made during the last six months to obtain such a method I have only been able to obtain partially developed parasites in one instance from mixtures of finger-blood and sterile citrate of sodium solution, drawn into sterile pipettes, which were sealed and incubated at 20° C.

Examinations of the blood of a kala azar patient now in hospital here have shown that it is only possible to find the Leishman body if a prolonged and exhaustive search, involving the careful examination of some 400 or 500 white blood cells, is made; sometimes even this fails. In any case, in the leucopenia generally present in kala azar, one must examine nearly half a c.m.m. of blood before one can hope to find Leishman bodies; separation of the white blood cells by centrifugation, or allowing the citrated blood to stand, helps to diminish the difficulty, but does not give really satisfactory results.

The difficulty in obtaining the easily recognisable developed
forms from incubated citrated finger blood may be due possibly to
an increased antiparasitic power of the peripheral blood over splenic
blood, as well as to paucity of the parasites in the peripheral
blood.

(3) The Leishman body does not appear to be eliminated in the
faeces or urine, even when present in the intestine and kidney, and
if the negative results recorded in this case were partly due to the
obvious difficulties met with in searching films from faecal dilutions,
the parasite certainly does not develop out in cultures of faecal
dilutions incubated at 20° C., for if present the developed forms of
the Leishman body would easily be recognised. The parasites in
the kidney were so scarce that no similar conclusion can be
definitely arrived at with regard to the urine—but here, again, no
Leishman bodies were ever found in the urine and no development
took place in incubated dilutions of urine.

(4) The Leishman body appears capable of partial development
in the body after death in certain circumstances.

(5) The distribution of the parasite in the tissues is a wide one,
but the intensity of the infection varies strikingly; organs like
the liver, spleen, bone-marrow and lymphatic glands, if infected
contain enormous numbers of Leishman bodies. Other organs,
even when infected, contain usually very few parasites. This inten­
sity of parasitic infection in such organs as the spleen, bone-marrow,
liver and lymphatic glands is probably due to their filter-like
nature.

(6) The parasite is found in unchanged or swollen endothelial
cells. It is also found in reticular cells and myelocytes, in large
mononuclear and in polynuclear white blood cells. I have never
seen a Leishman body in a red blood cell, glandular cell, or free in
the blood. The darker staining reaction of some of the endothelial
cells which contain large numbers of parasites may be due to
changes in the cell protoplasm produced by the presence of many
parasites.

(7) The primary infection of a tissue is probably brought about
by a parasite enclosed in a white blood cell (large mononuclear
generally.) The spread of the infection in that tissue is probably
partly due to cell-to-cell infection, as suggested by Christophers.
The endothelium of the large blood-vessels is almost invariably free
of parasites, while they are generally found in numbers where these
larger vessels break up into their ultimate capillaries; this condition
suggests that the parasite or parasite-holding white blood cell can­
not be taken up by the phagocytic vascular endothelium from the
swifter flowing blood in these larger vessels, but is dealt with in the sluggish stream of the capillary or reticular space.

(8) There is no direct microscopic evidence either in this case or that of R—, previously reported, in favour of infection primarily through the intestine and portal pathway, while the portal zones of the liver lobules were slightly more infected by parasites than the hepatic zones; this might be due to the treble liability to infection of these areas by the hepatic artery, portal vessels and lymphatics.

The splenic pain and enlargement in W—’s case also preceded similar liver symptoms by about a week, but this fact is not of much value, for the spleen is enlarged in typhoid without the liver being affected, and here the bacterial invasion of the body is probably by the portal pathway.

The study of this case and that of R—'s, reported in the January and March numbers of this Journal, was partly undertaken in order to gain, if possible, some clue to the way in which the human body is primarily infected by the parasite, and I hope it will not be considered out of place if this question is briefly discussed. The two most likely modes of infection are:

(1) By the alimentary tract, through water or water animalcula.

(2) Infection through the skin (by blood-sucking insects, leeches, &c.).

In favour of the first view we have the close relation between the spleen rate and water supply in some parts of India, and Lieutenant McKenzie of our Corps writes, that around Dum Dum, where a large proportion of the big spleens are probably due to kala azar, the spleen rate is closely associated with the water supply; the purer the water supply, the less the big spleens in a district. At first sight, also, the intense nature of the parasitic infections of its organs (liver and spleen) in close relation with the intestinal tract and the frequent infection of this tract itself, in many cases, would appear to render support to the theory of infection by the gastro-intestinal tract. The intestine, however, is not always infected. Rogers says he found practically no intestinal ulceration in his Assam cases, while in the two post mortems on kala azar held here the intestinal infection in the present case (W—’s) was very diffuse and slight, and in R—’s case not only were no macroscopic or microscopic changes seen in the intestine, but the mesenteric glands were not enlarged, and no parasites found in them on microscopic search. The infection of the intestine in kala azar,
A Case of Kala Azar

even when definite, could be due to a local deposition from parasite-holding blood cells in the general circulation. Such a deposition undoubtedly occurs in the skin.

Further, it must be remembered that the intestinal tract is peculiarly liable to slight inflammations and superficial ulcerations, and this condition of blood stasis in and extravasation from the blood capillaries of the part would produce conditions particularly suited to the deposition of the Leishman body in these inflamed areas of intestine. The slightly greater intensity of infection of the portal areas of the liver lobules might be cited in favour of the intestinal theory, but the triple possible source of infection of these areas already commented on might account for the condition.

If the parasite does enter by the gastro-intestinal tract it, in all probability, does not do so through water itself, but in the body of some water animalculæ, for all my attempts to cultivate the parasite out in water have failed. The parasite, on the contrary, readily dies and degenerates under these conditions. It does not seem reasonable even, that a parasite which finds water so unfavourable a medium should have one of the stages of its life-history in the body of any water animalculæ. The spread of kala azar through infected feces or urine does not appear likely in face of the continued failure (months of search) to find either Leishman bodies or their developing forms in faecal cultures of highly diluted feces and urine incubated at 20° C.

Further, the parasites readily die in bacteria-infected cultures. The other likely source of infection is through the skin, the parasite being conveyed through the bite of some blood-sucking insect, leech, &c., and there is much to be said in favour of this view.

(1) The disease has been shown by Rogers to be epidemic in the rainy season in Assam. A period when insect life (leeches also) is much increased.

(2) The same observer has just brought to notice three instances of healthy white men having contracted kala azar after association with three women suffering from the disease and who eventually died of it. As the women here probably visited the men, the infection could scarcely have been through water, though I must say it is a disease with a probably long incubation period. With kala azar the infection of three people, and in a district like Assam where the disease is endemic, is not much to go upon.
(3) Kala-azar, when prevalent in one range of coolie lines in plantations in Assam, appears to be readily stamped out by moving the healthy coolies to new lines. As these moves apparently take place on the same plantation, it is extremely unlikely that in all cases a new water supply has been obtained for the coolies.

(4) The Leishman body develops readily up to 27° C. (i.e., 80° F.) in artificial cultures. This temperature is not far removed from that of the interiors of houses, and of shady spots in Assam during the rains. These situations are favourite insect resorts.

(5) Rogers has just shown that the optimum medium for development of the Leishman body is an acid one. The stomach contents of many insects are apparently slightly acid; and the same observer, though failing to get developments in the bodies of fleas and bugs, yet finds that Leishman bodies retain their vitality for several days in the blood-filled stomachs of these insects.

The evidence derived from cultivation experiments is thus against the water theory, while there is no direct evidence in favour of it. There is one fact which somewhat militates against the theory of spread of the disease by skin bites, and this is that the parasite is so rarely found in the peripheral circulation in the cases we see at home; but this may not be the case in the earlier phases of the disease; in fact, Christophers has found 37 parasites in 500 white blood cells in one case, and 9 in a similar number of cells in another in two cases in India. An infection of the peripheral circulation of this degree could present no difficulties in the transference of the disease through insects.

If the parasite enters through the skin it probably does so in the shape of a small spirillum contained in the saliva or other secretion of some blood-sucking animal. The spirillum once in the human tissues would probably soon change its shape owing to the high temperature of the body, &c., to the small encapsuled-like form known as the Leishman body. It would then probably be taken up by a white blood cell (usually large mononuclear), be swept into the general circulation and lodged in one of the filter organs, spleen, liver, bone-marrow, &c. In the sluggish circulation of, say, the spleen lymph space, the parasite-containing cell would be taken up by the phagocytic endothelium of the spaces, and once in this cell the parasite would rapidly multiply, and mainly through cell-to-cell infection the entire organ would become infected. Parasites from the infected organ could be carried to and
A Case of Kala Azar

lodged in the ultimate capillaries and tissue spaces of other organs and tissues, such as the liver, bone-marrow, skin and intestine; especially if there was any slight ulceration or inflammation present in these latter tissues favouring vascular stasis and cell extravasation. The disease would be now well established, and the kala azar patient would become in his turn an infective agent. If the parasites of this man were now taken up by a blood-sucking animal, the changes described by Colonel Leishman of increase in size, flagellation, and splitting off of smaller and smaller trypanosome and spirilla-like forms would take place in the tissues of the alternate host, till the ultimate spirilla, perhaps ultra-microscopic, were formed and transferred to a new human host.

The partial development of the Leishman parasite in the human body after death is interesting, and would be worth further observation, but I do not think that a transference of the disease from dead bodies is likely, as the parasite dies so readily in infected cultures.

LITERATURE.

CHRISTOPHERS. "Scientific Memoirs by Officers of the Medical and Sanitary Services with the Government of India," Nos. 8 and 11 (new series), 1904.


LEISHMAN and STATHAM. Journal of the Royal Army Medical Corps, March, 1905.

STATHAM. Journal of the Royal Army Medical Corps, January, 1905.


MARCHAND and LEDINGHAM. Zeitschrift für Hygiene und Infektionskrankheiten, 1904.

WERIGO. Annales de l’Institut Pasteur, 1892.


MCKENZIE. Journal of the Royal Army Medical Corps, October, 1904.