A COMPARATIVE STUDY OF EXPERIMENTAL BILHARZIASIS IN MONKEYS CONTRASTED WITH THE HITHERTO DESCRIBED LESIONS IN MAN.[1]

By N. HAMILTON FAIRLEY, M.D. (Melb.); M.R.C.P. (London); D.T.M. & H. (Camb.), O.B.E., *Lieut.-Colonel A.A.M.C.; late Pathologist, 14th Australian General Hospital; Pathologist, Walter and Eliza Hall Institute of Research, Melbourne.*

(PLATES X.–XIV.)

I.

SCOPE OF THE PRESENT INVESTIGATION.

IN the months of August and September 1916 Lawton (1917 [1]) noted certain obscure clinical symptoms in twenty-four early cases of bilharziasis admitted to No. 3 Australian General Hospital, Cairo. The clinical syndrome which supervened four to twelve weeks after exposure to infection, consisted of a febrile attack accompanied by abdominal pain, enlarged tender liver and spleen, bronchitis, urticaria and perhaps diarrhœa. Microscopical investigation showed both the presence of a high eosinophilia and lateral spined ova of *B. mansoni* in the fæces.

In the following months of the same year a similar history was obtained from a large percentage of cases admitted to the 14th Australian General Hospital suffering from bilharziasis. I also found a similar symptom-complex in cases infected with *B. hæmatobia* alone. Since, fortunately, owing to the limited number of exposures to the infection, none of the cases proved fatal, no human pathological material was available for investigation. I therefore decided to make a complete study of early bilharziasis as it occurred in experimentally infected monkeys, hoping thereby to establish a pathological basis for the early clinical picture as manifested in many of these human cases.

During the subsequent two years (1917 and 1918) twenty-five monkeys have been experimentally infected with one or other variety (*B. mansoni* or *B. hæmatobia*),—their symptoms noted, their morbid

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anatomy and pathological histology studied at varying periods within the first few weeks of infection, and the nature of the cellular-humoral response investigated.

Where possible a comparison with the human pathology of the more advanced stages of the disease has been made.

II.

THE ARTIFICIAL INFECTION OF MONKEYS WITH BILHARZIASIS.

Subsequent to Bilharz's discovery of the parasite in man it was Cobbold who first demonstrated similar worms in the portal vein of the African Sooty monkey (*Cercopithecus fuliginosus*). The fact that a natural infection with these trematodes can occur, together with the close biological relationship of these animals to man, makes monkeys particularly suitable for such experimental work. Of the twenty-five monkeys two were infected by the upper alimentary tract; the remainder by the cutaneous or subcutaneous routes either alone or combined with an alimentary infection. In order to infect the animals by the *cutaneous* route the following methods were adopted: either water containing cercariæ was poured over the floor of the cage or an area of skin was shaved and on it was placed a teased out preparation of the liver of an infected snail. The whole selected area of skin was then kept moistened for twenty or thirty minutes by dropping water from a pipette. In order to produce infection viâ the *subcutaneous* route 10 c.c. of distilled water containing cercariæ derived from a heavily infected liver were injected subcutaneously.

In producing infection by the alimentary canal the drinking water was contaminated with infected Planorbis snails. Frequently it was found necessary to increase the salt content of the dietary or to withhold water for twenty-four hours in order to increase the monkey's thirst. Leiper (1915 [2]) has shown that hydrochloric acid in a dilution of 1 in 1000 is lethal to cercariæ; they must therefore invade the buccal, pharyngeal or œsophageal mucosa before reaching the stomach, if infection by this route is to ensue, as they would otherwise be killed by the gastric juice. That invasion by cercariæ causes irritation is shown by the frequency with which monkeys are observed to scratch their skin and lips during contact with infected water.

Leiper has also shown that in the process of penetrating the skin the cercariæ cast off their tails, which are therefore merely larval structures; thence they bore their way into the corium, where they invade certain venules and ultimately reach the portal veins of the liver. Here they develop, mate and attain maturity. The passage of cercariæ viâ the lymphatic system must, for merely anatomical reasons, be a much less desirable or frequent route of reaching the organ.

III.

THE SYMPTOMS PRODUCED IN EXPERIMENTALLY INFECTED MONKEYS.

At a variable time after infection, *i.e.* from the second to the sixth week, some or all of the following symptoms develop :—

Emaciation, muscular weakness, anorexia and perhaps rigors. In hyperinfected animals death ensued with intensification of these symptoms associated, perhaps, with malæna. In the animals which survived this period these systemic symptoms subsided and, after a quiescent period, those of localised bilharziasis followed. In *B. mansoni* infections dysenteric symptoms supervened from the sixth to the eighth week, but in *B. hæmatobia* vesical ones were not noted till a later date. Indeed, in these latter cases, terminal spined ova could be demonstrated earlier in the fæces than in the urine.

The explanation of the late appearance of symptoms in *B. hæmatobia* infection would appear to lie in the greater distance the adult worms have to travel before reaching the bladder.

IV.

OBSERVATIONS ON THE ADULT PARASITES AND THEIR OVA.

In order to correctly appreciate the method of the production of pathological lesions in the two infections a careful study of bilharzia worms and their ova is desirable.

(A) The Mode of Deposition of Ova in the Tissues.

It is remarkable that the exact mechanism involved in the deposition of ova in the tissues has never been determined. Perhaps Looss (1908 [3]) came nearer to the solution of the problem than other workers, but even he had only discovered part of the truth. Considerable attention has therefore been devoted to this interesting aspect of the pathology of the disease, and the following description is based entirely on a series of personal observations.

The different stages in the process may be enumerated as follows :—

1. *The Movement of the Parasites within the Venous System.*

Examination of the adults of *B. mansoni* and *B. hæmatobia* in the mesenteric veins of anæsthetised monkeys has shown that they progress by peristaltic-like movement of the body and by the action of the ventral sucker. Females may occasionally be seen by the aid of a hand lens alone in veins of smaller calibre, and then they appear as sinuous, dark, elongated, thread-like structures. This dark appearance is due to the presence of digested hæmoglobin in their intestinal cæca.

2. *The Distension of the Walls of the Venule by the Female causing Temporary Stoppage of the Blood Current.*

When the time for egg-laying is at hand the female leaves its male partner and progresses against the portal stream to the smaller venules. By a process of elongation and in virtue of its great muscular power the female worm works her anterior truncated extremity, or indeed her whole body, into a vessel of smaller diameter than her own, thus dilating the vessel wall. Measurements from dead specimens by other observers have shown that the diameter of the anterior truncated portion of the female worm is 100 μ, and that of her body 274 μ. In life, however, these diameters may be further reduced. Serial sections of a heavily infected organ will occasionally demonstrate a female bilharzia worm in close opposition to the walls of a venule, distending it so that the muscular coats are stretched and thinned. Naturally the effect of such complete plugging of a vessel is to obliterate temporarily the venous flow.

3. *The Mode of Deposition of Ova in the Venules.*

After distending the venule the female worm ejects an ovum from the genital pore which is situated just posterior to the ventral sucker. Its blunt conical end is directed anteriorly against the venous flow and its posterior end bearing the spine (lateral or terminal) points backwards in the direction of the blood current. The cause for the position assumed by the ovum is obvious if one examines carefully the uterus of the gravid female worm, for here also ova lie in this same position.

4. *Contraction of the Distended Venule on the Contained Ova.*

In the next stage the female withdraws slightly, leaving the ovum deposited in front; at the same time the over-distended venule tends to resume its normal calibre by contracting down upon the ovum. Another egg is then deposited and again the female withdraws a little; simultaneously contraction of another segment of the venule ensues, and thus the process continues. Only by this hypothesis can I explain the appearance of a line of ova distributed at regular intervals dilating the vessel in nodular fashion and resembling, more than anything else, a string of miniature sausages (Plate XII. Fig. 5).

Direct microscopic examination of pieces of the small intestine of monkeys squeezed between two glass slides by means of rubber bands has revealed many interesting features.

1. Lines of ova can be seen distending venules of a diameter less than their own, so that the appearance of "strings of sausages" is simulated.

2. Such ova lie with the blunt conical end directed away from the main trunks and the spined end pointing in the direction of the venous flow.

3. The various stages in the egress of ova into the perivenous tissues can be traced; firstly, the spine in contact with the wall; secondly, the piercing of the coat; thirdly, the passage of ova through the resulting tear; fourthly, their deposition around the vessel.

4. The greatest number of lateral spined ova in one vein has been six, but as many as twenty terminal spined ova have been observed. Rarely the ova of *B. hæmatobia* are packed in the vein in pairs, and in such cases it is possible that they were ejected progressively in front of the female worm in contradistinction to the more usual mode of deposition I have described.

Such a condition cannot be produced by the progressive ejection of ova in front of the female worm, though occasionally deposition in this fashion is possible, as will be shown later.

5. *The Withdrawal of the Female and the Resumption of the Blood Current.*

When the female has deposited her last egg and has withdrawn altogether from that particular branch, the blood current tends to force its way again through the venule containing the deposited ova. In so doing the blood impinges on their blunt conical ends, and, unless the long diameters of the ova lie parallel in a mathematical sense to the walls of the venule, the ova will be turned in an oblique direction.

6. *Passage of the Ova through the Vessel Walls into the Perivenous Tissues.*

It will be remembered that these ova, as observed under the microscope, are deposited in venules with their spines pointing in the direction of the venous current. Any movement must, in consequence, result in their spines engaging in the vessel wall. Obliquity, in the case of the terminal spined ovum, ensures this result, while the position of the lateral spine in *B. mansoni*, increasing as it does the transverse diameter of the ovum, does likewise. Once the spines have engaged the vessel wall and pierced it, the force of the venous blood stream will tear a rent in the venule and drive the ova into the perivenous tissue. The spines, hereafter, take no part in the process of extrusion of ova through the tissues to the exterior, which is dependent on the inflammatory softening and cellular accumulations in its vicinity.

It should be here mentioned that where ova are deposited in vessels of larger calibre (as sometimes occurs in *B. hæmatobia* infections where terminal spined ova occasionally are observed impacted in pairs), over-distension and subsequent contraction of the venule may not take place. All the other factors described, however, are operative, and thereby most of the ova successfully gain the perivenous tissue. Occasionally ova will escape, which are filtered out mainly in the portal veins of the liver in the case of *B. mansoni*, and in the lung and liver

in the case of *B. hæmatobia*. Escape into the perivascular tissues in these organs is produced by mechanical rupture by ova impacted in the vessels, the spines playing but a minor rôle in the process. Needless to say this latter explanation applies to all ova which reach the viscera (heart, kidney, etc.) by the systemic vessels.

(B) The Ova-producing capacity of *B. hæmatobia* and *B. mansoni* contrasted.

In *B. hæmatobia* the uterus is situated in the posterior portion of the female worm and is of much greater size than is that of *B. mansoni*, in which it is situated in the anterior half of the body. In consequence *B. hæmatobia* has a much greater capacity for storing ova, and probably for producing them, than has *B. mansoni*. Leiper (1915 [2]) states that *B. mansoni* contains only one lateral spined ovum. While this certainly is the rule, I have repeatedly seen three in the uterus of the same female, and, during direct examination of the small intestine of a heavily infected monkey, six lateral spined ova were once observed in the one venule—presumably all deposited in succession by the one female worm. In *B. hæmatobia* many terminal spined ova occur simultaneously *in utero*; as many as fifty have been counted. I have also observed twenty terminal spined ova impacted in pairs in one venule of the small intestine. Such observations are important inasmuch as they afford a partial explanation of the latency of the symptoms and the more benign course of *B. mansoni* infections.

In the Delta the sweet-water canals in many regions contain many more snails of the species *Planorbis boissyi* than of *Bullinus dybowski contortus*, or *innesi*. Furthermore, Bahr and the writer found 18·0 per cent. of the former infected throughout the whole year and only 1·9 per cent. of the latter. On the other hand, in Egypt the most commonly recognised clinical symptoms are referred to the bladder, and therefore are infections with *B. hæmatobia*. Unless there is some other intermediate host for the latter, the explanation for this phenomenon must lie in the greater latency and more benign course of *B. mansoni*, for undoubtedly this infection is very widespread amongst the fellaheen of Egypt.

(C) The Habitat of Adults of *B. hæmatobia* and *B. mansoni* in the Body.

During the present investigation living worms have never been found outside the vascular system. Indeed, in microscopic sections of an organ, such as the lung, in which recently defunct worms were occasionally observed surrounded by a dense eosinophile infiltration and lying apparently free in the tissues, a more careful examination always revealed the outlines of the containing blood vessels. There can be no doubt that the adult worms are parasites of the blood vascular system only, and for this reason intravenous therapy in the administration of parasiticidal drugs is indicated. After developing

and maturing in the portal veins of the liver, many of the paired worms migrate against the portal blood stream into certain of the abdominal and pelvic veins, but whether they are guided there by instinct or some obscure chemiotactic stimulus is unknown. Naturally ova which are deposited in the submucous tissue of the hollow viscera, such as the intestine, bladder or ureters, have every chance of reaching the external world in a short time. Numbers of ova, however, are continually being lost in the tissues. Even a proportion of those deposited in favourable situations for extrusion are destroyed or imprisoned by vascular and tissue reaction, while those deposited in the subserous zone of the hollow viscera, as well as in the solid organs like the liver and kidney, can never attain their physiological destiny.

The location of bilharzia worms, as ascertained by autopsy performed immediately after death, shows that the distribution of the parasites in the body is somewhat different for the two species. In *B. mansoni* infections the parasites are mainly found in the branches of the *portal*, the *superior* and the *inferior mesenteric veins.*

In *B. hæmatobia* infections the parasites are also found in these veins, but can be demonstrated in the greatest numbers in the *pelvic plexuses, especially the prostatic, vesical and uterine.*

These situations are reached by travelling viâ the inferior mesenteric vein to the inferior hæmorrhoidal plexus, which communicates directly or indirectly with those mentioned above. As these plevic plexuses drain into the inferior vena cava, it is not surprising that adults of *B. hæmatobia* are found in the pulmonary arterioles.

It is noteworthy that in both species of infection these parasites rarely inhabit the splenic vein.

(D) The Distribution of Ova in Various Organs in Infected Monkeys.

In estimating quantitatively the number of ova deposited in any given organ a modification of Ferguson's (1913 [4]) method was adopted. Approximately equal weights of various tissues were divided into small blocks and digested for 24 to 72 hours in 3 per cent. caustic soda at 37° C. The mixture was then centrifuged and the number of ova contained in smears on the sediment roughly estimated. While a fairly general distribution of ova in tissues was established for each species of infection the maximum incidence of ova deposited varied considerably.

Thus in *B. mansoni* infection the *greatest number of ova were always found in the colon, liver and small intestine.* They were also demonstrated in the stomach, duodenum, gall bladder, pancreas, spleen, bladder, lymphatic glands and lung. More rarely a few lateral spined eggs were found in the kidney, suprarenal gland and the myocardium.

In *B. hæmatobia* infections the maximum deposit of ova occurred in the *pelvic viscera, especially the bladder and uterus.* Large numbers

were found constantly in the liver, small and large bowel and the lung. They were also demonstrated in the lymphatic glands and pancreas.

A quantitative survey of the ova content of the various organs and tissues leads to the conclusion that, as a rule, *a definite relationship between the production of macroscopic lesions in the viscera and the quantitative distribution of ova in these situations exists.*

The possible exceptions are the lungs and liver, where toxins as well as ova are being filtered out. This relationship is well illustrated by the naked-eye changes in the colon in infections with *B. mansoni*, or in the bladder, uterus and colon with *B. hæmatobia*. On the other hand, I have never noted macroscopic lesions in the spleen and only once in the pancreas of infected monkeys. Wherever ova are deposited in the tissues naturally focal lesions do occur, but if they are few in number then the lesions are of microscopic size and remain undetected.

Wherever ova are deposited in the solid viscera (*i.e.*, spleen, pancreas, liver, lung, kidney) it is either an accidental or an exceptional occurrence.

(E) The Focal Distribution of Ova within the Various Organs.

The chief factor determining the focal distribution of ova in any organ is the anatomical distribution of the blood supply and the calibre and elasticity of its vessels.

In the hollow viscera, such as the intestine and bladder, it can be demonstrated in microscopic sections that ova and worms are generally located in the submucous or subperitoneal zones, while the muscular layers are unaffected. Occasionally ova may be seen between the circular and longitudinal muscular coats, and then always in the inter-muscular fibrous tissue. Adult worms may be actually encountered in the veins lying within the outer muscular layer of the uterus. Letulle (1905 [5]) has suggested that the female worms avoid the muscular layers of the hollow viscera owing to the pressure and constant contractions taking place in this situation. In the lung the distribution of ova occurs around the pulmonary arterioles, and in the liver around the branches of the portal veins. In the systemic viscera—lung, heart, muscle, etc.—they occur in the vicinity of the terminal arterioles.

V.

THE PATHOLOGICAL LESIONS IN EXPERIMENTALLY INFECTED MONKEYS AND MAN CONTRASTED.

(A) The Pathological Picture in Monkeys dying within Five Weeks of Infection.

All these animals were infected repeatedly by the cutaneous route with cercariæ of *B. hæmatobia* derived from *Bullinus dybowski*.

The three from which this study has been made died on the fifteenth, twenty-second and thirty-fifth day of the disease.

These monkeys must have died as a result of a toxæmia for the following reasons: paired worms do not leave the portal vein till the fourth week after invasion and ova are not deposited in the tissue till the fifth. This subject will be referred to later in more detail. At autopsy the spleen was diffluent and liver and kidney congested and swollen; in the latter two the cells showed signs of cloudy swelling. The lungs were congested at the basis and in two cases petechiæ were seen in the colon.

The blood showed a leucopenia and a severe secondary anæmia.

THE MICROSCOPIC PICTURE OF THESE ORGANS WAS AS FOLLOWS:—

1. *Liver.*—The liver showed acute venous congestion with cloudy swelling of the parenchyma and actually, in the more chronic cases, displacement of the hepatic tissue by focal collections of inflammatory cells (Plate X. Fig. 1*a*).

These were composed of mononuclear and polymorph cells, especially in the periportal areas, suggesting the presence of a circulating toxin. No traces of bilharzial pigment were seen at this early stage.

2. *Spleen.*—The spleen was the site of extreme congestion leading to attenuation of the splenic pulp, thus causing Malpighian bodies to appear prominent in microscopic sections in the midst of the general congestion; small intra-reticular hæmorrhages were occasionally noted. Often a finely granular dark brown pigment occurred both intra-cellularly and extra-cellularly situated.

3. *Kidney.*—The cortical epithelium was swollen and the cytoplasm in process of disintegration. The lumina of the tubules were filled with a finely granular material or by albuminous coagula and rarely by actual hæmorrhage.

Enlargement of the glomeruli due to congestion or to cellular infiltration was also present with inflammatory changes in the afferent tubules.

4. *Lungs.*—The lungs showed nothing of interest beyond passive congestion and alveolar catarrh.

Such changes are undoubtedly indicative of an acute toxæmia, the brunt of which is falling on the parenchyma of the liver, the spleen and the cortical epithelium of the kidney.

Hyperinfections of such magnitude have never been recorded in man, therefore I have not been able to make a comparative study as I have done in other sections.

(B) THE PATHOLOGICAL PICTURE IN MONKEYS DYING AFTER THE SIXTH WEEK OF INFECTION INVESTIGATED WITH THE OBJECT OF ESTABLISHING A BASIS FOR THE EARLY TOXÆMIC SYMPTOMS WHICH HAVE BEEN OBSERVED IN MAN.

In monkeys coming to autopsy from the sixth to the fourteenth week of the disease, the pathological picture differs considerably from that already described. Focal lesions of bilharziasis, which bear a very definite relationship to the intensity of the infection—that is, to the number of paired worms attaining sexual maturity within the body of the definitive host—now appear. Such lesions are dependent

on the deposition of ova in the tissues as well as upon the action of the circulating toxin.

Each species of bilharzia (*B. hæmatobia* and *B. mansoni*) produces an identical type of pathological lesion. The only essential difference in the pathology lies in the regional distribution of the lesions. Therefore the distribution of the macroscopic lesions in the two infections is regulated by the habitat of the adults of each species.

In *B. mansoni* infections the adult worms are found in the portal veins of the liver, and in the inferior and superior mensenteric veins. The macroscopic lesions involve the liver, colon and sometimes the small intestine.

In *B. hæmatobia* infections, whilst the adult worms also inhabit the portal veins of the liver and the superior and inferior mesenteric veins, they appear to have a real predilection for the pelvic plexuses which drain into the inferior vena cava. In addition to involvement of the liver and colon in this infection, one also finds lesions in the pelvic organs such as the bladder and uterus, and also, not uncommonly, in the lung.

Before studying in detail the pathology of the various organs involved in bilharziasis (*B. hæmatobia* or *B. mansoni*) it would be well to consider briefly the general types of lesions produced. Within three months, in heavily infected monkeys, true adeno-papillomata may develop (especially in *B. hæmatobia*), but undoubtedly the most common lesions observed are pseudo-tubercules, or small whitish nodules of various sizes of from 0·5 mm. to 4 mm. in diameter, which indeed may have, especially in *B. hæmatobia*, a widespread distribution.

1. *The Structure of a Typical Pseudo-Tubercle.*

This is very characteristic. In the centre may be found one or more ova with terminal or lateral spines and crenated chitinous shells and containing myracidia. Giant cells or a plasmodial mass tending to envelop the ova are frequently present at this early stage. Surrounding them there is a zone of polymorpho-nuclear eosinophil cells and more peripherally placed a similar zone of mononuclears. Naturally the appearance of such a focus will vary according as to whether the section passes directly through the centre or not.

In the most severe infections cells may be so crowded together, and the absorption of toxins locally produced so great, that degeneration of the centrally placed eosinophils may ensue. The resulting amorphous material, staining diffusely with eosin, contains pyknotic nuclei scattered throughout its substance. Subsequent to these evidences of tissue destruction, reparative changes sooner or later eventuate. These are indicated by the appearance of encircling fibroblasts and of linear capillaries budding in from the periphery at right angles through other and more centrally placed cells.

As these changes progress the eosinophils tend to decrease in numbers or to disappear, leaving the mononuclear cells in evidence. These changes coincide with the passage of the ova containing live myracidia, which either escape from the tissues almost immediately, or, becoming encircled by fibrous tissue, are held up indefinitely with the consequent death of the contained embryos. The late degenerative changes in the bilharzia ova were not seen in any of the

experimental monkeys, though they occur frequently in human tissues which have long been the seat of the disease.

1. GIANT CELLS.—These cells are produced in response to mechanical irritation by the ova and are probably of endothelial origin. They are often of a large size with peripherally placed oval nuclei in active karyokinesis; even individual cells may coalesce to form a plasmodial mass, the largest of which may measure 120 μ in length, and such a mass may enclose an intact ovum.

2. EOSINOPHIL CELLS.—During the course of this pathological study I have been able to confirm Day's (1911 [6]) experimental work with extracts of ova and worms injected subcutaneously by finding a local eosinophilia in the immediate vicinity of ova and adults, as well as an actual increase of these cells in the lumina of adjacent vessels which appear to be packed with them; their nuclear activity is shown by the karyokinetic changes (equatorial bar) which they exhibit.

A study of the peripheral blood of these monkeys during life has shown a leucocytosis together with a relative, as well as an absolute, increase in the eosinophils, and at the same time a decrease in the neutrophil polymorphs.

A study of the *bone-marrow* shows the same activity of the eosinophil elements, both in the myelocyte as well as in the fully developed stage. It would appear then that, not only do the adult parasites, but also the myracidia contained in the deposited ova, excrete a specific toxin which exerts a chemiotactic action upon the eosinophil leucocyte and stimulates their production in the bone-marrow.

It is only in this acute stage of the disease that such great eosinophil accumulations are seen; it is otherwise in chronic cases—as in human tissues—where the ova are mostly dead and partly calcified and are encapsulated in fibrous tissue in which eosinophil cells are rare; one may assume that it is the living myracidia situated within the ova which evoke an eosinophil response. In human tissues, particularly when collected at operation, I have seen similar eosinophil collections round living ova.

3. SMALL ROUND CELLS.—These inflammatory cells occur commonly in recent bilharzial lesions; these appear to be derived partly by diapedesis and partly by proliferation of connective tissue.

4. PIGMENTED CELLS.—Not uncommonly pigmented cells were noted in the vicinity of bilharzial lesions. In the liver this pigment occurs within Küpffer's endothelial cells, but in other organs a similar one is found within the mononuclear leucocytes.

As regards its nature it appears to be closely similar to, or identical with, malarial pigment, and indeed it exists in the intestinal cæca of the adult parasites and is derived from blood metabolised in the process of digestion. Whether this is the sole source, or whether it originates in the hæmolytic action of the bilharzial toxins, still remains to be decided.

A more detailed description of the physico-chemical characteristics are given under heading 2 (*infra*).

2. *The Pathology of the Liver* (B. hæmatobia *and* B. mansoni *infections*).

The constant morphological changes observed in the liver of experimental monkeys in both kinds of infection are due to the maturing parasites, whose habitat is the portal vein and its tributaries, whence the circulating toxins are filtered out into the periportal zones. The remarkable fact is that a similar involvement of the liver has not been more constantly described in human bilharziasis.

The liver is enlarged, dark and congested; the glandular substance, as well as the capsule, is beset with round or oval pale-coloured nodules 0·5 to 3 mm. in diameter (Plate XIV. Fig. 9). These nodules resemble tubercles, but easily break down on pressure, with the exudation of a creamy mass composed of disintegrating eosinophils.

The bile ducts themselves have never been observed to be affected, but occasionally similar cellular accumulations may occur in the gall bladder, and the bile itself contain viscid mucus and characteristic ova which have found their way into the viscus by the central softening of such a nodule. Adult bilharzia parasites are found sometimes in large numbers in the portal vein (Plate XIII. Fig. 7), from which they may be dislodged by compressing the liver at autopsy. In monkeys these pathological lesions are well established six to eight weeks after infection, *a period which synchronises exactly with the clinical picture of acute bilharziasis in man and which is associated at this stage with a large and tender liver.*

The Microscopic Pathology of the Liver.—Cellular accumulations round ova, as I have already described, are scattered throughout the substance of the liver (Plate X. Fig. 1*b*), especially in Glisson's capsule and the periportal zone. The exudation of eosinophil cells is such that islets of hepatic cells may be compressed and actually undergo atrophy (Plate X. Fig. 1*c*). The liability of these cell accumulations to central degeneration has already been commented on, but later the establishment of a cirrhosis can be studied in all its details as follows:—firstly, the appearance of fasciculated fibroblasts dividing the eosinophil leucocytes into separate masses; secondly, the appearance of freshly formed capillaries; and thirdly, the envelopment and isolation of islets of hepatic cells, the nuclei of which may be so massed together as to resemble giant cells, as in other cirrhotic processes cell islets formed of atrophied cell elements (pseudo-bile ducts) make their appearance. Congestion of the interacinar capillaries may be generalised or localised, and in the latter case may be sufficiently intense to lead to compression of the hepatic parenchyma, but quite apart from these effects of pressure the cells exhibit an indistinct outline with granular endoplasm—the stigmata of a diffuse toxæmia.

Bilharzial Pigment.—A constant feature is the presence of a rich ochreous or golden brown *pigment* in the endothelial cells of Küpffer and is common to both types of infection.

It closely resembles *malarial pigment* in its physico-chemical properties, and indeed all my observations go to show that the two pigments are identical. The bilharzial pigment does not give the Prussian-blue reaction with the potassium ferrocyanide test and is insoluble in 5 per cent. hydrochloric and sulphuric acids.

It is soluble in—

1. A saturated aqueous solution of lithium carbonate.
2. A 0·2 per cent. aqueous solution of potassium hydroxide.
3. Alcoholic potash.
4. Acid alcohol (1 per cent. HCl in 70 per cent. alcohol).
5. Hydrochloric acid ether (1 per cent. HCl).

My reason for emphasising its identity with malarial pigment is founded on the excellent work of Brown (1911 [7]), who has demonstrated similar reactions for malarial pigment by which it appears more than probable that "hæmozoin" is not melanin, as is generally held, but "hæmatin," a direct derivative of hæmoglobin.

In the portal veins, in suitably fixed tissues (as for instance with perchloride of mercury), paired parasites may be demonstrated in surprisingly large numbers, but I have never observed any proliferation of the endothelium of these vessels either in monkey or human tissues.

The outstanding pathological features of the hepatic lesions in experimental bilharziasis are :—

1. In monkeys, the liver is invariably implicated in the early stages of infection.

2. Both parasites produce hepatic lesions, but from their anatomical situation, as might be expected, they are more intense in *B. mansoni* infections.

3. The scantiness of ova, as compared with the very extensive tissue changes, suggest that they are partly the result of a widely diffused bilharzial toxin which is present in the blood-stream and which is filtered out in the liver.

Bilharzial cirrhosis of the liver in man.—Impressed by the disparity in the hepatic changes of experimental bilharziasis compared with that described as occurring in man, Professor A. R. Ferguson and the author reinvestigated a quantity of material from this point of view. We concluded that no such disparity exists—that is, the human liver is much more constantly involved, even in the finer grades of cirrhosis, than has been previously supposed.

Our conclusions are :—

(*a*) The well-recognised type of pipe-stem cirrhosis, as originally described by Symmers (1904 [8]), is associated pre-eminently with lateral spined ova and represents the most advanced type, the result of prolonged and repeated bilharzia infection. Many intermediate stages exist associated with the presence of either lateral or terminal spined ova ; such lesions, though plain enough in microscopic sections, may be missed on naked-eye inspection.

(*b*) In human livers, recently deposited ova, as well as those containing defunct myracidia and which have undergone bipolar calcification, can be demonstrated, but nevertheless the tissue changes are out of all proportion, so that in this case also the generalised cirrhosis is the result of toxic action.

3. *The Pathology of the Large Intestine.*

The following description applies to the lesions produced by both forms, though owing to the predilection of *B. mansoni* for the inferior mesenteric vessels, the most marked intestinal changes occur in this infection.

The changes noted vary according as to whether ova are deposited in the submucous or subserous venules or in both.

Ova occur rarely in the muscular coats of the intestine ; when they do so, they are situated in the vicinity of the vessels of the intermuscular connective tissue, but the lesions they produce are of minute size.

In infected monkeys the whole large intestine, especially the

cæcum, transverse and descending colon, may be studded with large numbers of nodular cell accumulations; the base of these nodes is occasionally stained with a bluish discoloration (Plate XIV. Fig. 8).

On the intestine itself these nodules are grouped around the longitudinal muscular bands; the mesocolon is often involved also. A mild form of peritonitis may ensue with the formation of plastic bands and of omental adhesions.

The lymphatic glands draining the mesocolon in these cases are swollen, softened and enlarged. On incising the gut an acute dysenteric process is present, especially in the transverse and descending colon; the bowel contents being composed for the most part of blood and mucus.

The bowel wall is generally thickened and the mucosa intensely injected, with denudation of the epithelium. Tubercles are scattered on the free surface as well as early papillomata.

Microscopic Pathology.—(*a*) *Diffuse bilharzial dysentery* is much the most common manifestation during the first three months after infection and occasions numerous changes in the structure of the bowel wall.

The submucosa is greatly swollen by venous congestion and œdema; the predominant cell is again the eosinophil, and here and there red corpuscles escaping free in the tissues by diapedesis may occur. In the region of the muscularis mucosæ typical bilharzial cell accumulations—of giant cells, eosinophils and mononuclears—occur (Plate X. Fig. 2*a*). Adjacent to these collections the muscular fibres of the muscularis mucosæ may show hyaline and atrophic changes resulting from local pressure and toxic action.

Ultimately actual rupture of these fibres ensues and the contents of these minute abscesses break through into the *tunica propria*, thence through the basement membrane of the epithelium into the bowel lumen. By the formation and evacuation of these abscesses—for such they really are—actual ulceration of the mucosa takes place (Plate X. Fig. 2*b*). Secondary infection of the walls of these tracts leads to accumulation of neutrophil polymorphs in their vicinity, especially on the luminal side of the *tunica propria*. The adjacent columnar epithelium may undergo degeneration and exfoliation, but more usually individual cells show stigmata of unusual activity, both in nuclear division and in the secretion of mucus—thus explaining its abundance in the stools of early intestinal bilharziasis. Not every ovum which reaches the submucosa gains the lumen of the intestine; certain become held up by fibrous tissue and become localised.

Another feature of interest in the submucosa is the occurrence of spheroidal cellular masses with yellowish-brown pigment probably derived from the small interstitial hæmorrhages I have referred to.

In the *subserosa*, in addition to the pseudo-tubercles, definite changes may be in evidence, marked by the hyperplasia of connective tissue and fibrosis of the subserous fat.

In the most severe infections the subserosa may show all the acute changes noted in the other coats of the intestine. In the longitudinal bands of muscle, œdema of the lymph spaces and hyaline degeneration of the muscular fibres may be noted, but the actual occurrence of ova in this situation is rare. In the venules of both the submucosa and subserosa adult parasites may be noted, but they do not in any way seem to produce an endophlebitis of the mesenteric veins as described by Letulle (1905 [5]).

(*b*) *Formation of bilharzial papillomata.*—The earliest excrescences deserving of the name of papillomata have been observed in the colon within

ten to twelve weeks of infection. They are caused by the increased pressure exerted by massive cellular accumulations in the submucosa bulging up the *muscularis mucosæ*, the *tunica propria* and the glandular layer into conical elevations, and their core is always composed of submucous tissue. Excessive proliferation and downward invasion of the glandular tissue of the mucous layer, so characteristic of vesical papillomata of a similar age, have not been observed.

Bilharzial Lesions in the Human Colon.

The three types of lesions noted in monkeys may all occur in man. They may be classified as—

(1) Bilharzial pericolitis with subserous pseudo-tubercles, thus stimulating disseminated peritoneal tuberculosis.
(2) Acute bilharzial dysentery
(3) Adeno-papillomata of the colon.

1. *Bilharzial pericolitis resembling disseminated tuberculosis.*—These characteristic lesions which are so frequent in experimentally infected monkeys do not appear to have been recorded in man.

But since attention has been drawn to them Professor Ferguson has informed me that he has seen in a child of 6 years of age similar lesions throughout the colon, associated with a general enlargement of the mesenteric lymph glands. This case had actually been diagnosed on macroscopic examination as one of disseminated tuberculosis, until a microscopic examination proved its true nature, viz., cell aggregations round recently deposited bilharzia ova. Recently also I have seen a similar case with Dr. F. C. Madden in an appendix removed from a hernial sac, in which again it was not possible to determine its exact nature from a macroscopical examination alone. In this case also bilharzia ova were found.

The probable reason why this condition has not been more commonly recorded is to be found in the fact that, not being a fatal disease in man at this early stage, very few cases come to autopsy, save by accident or, when they do so, their real nature is not appreciated.

2. *Acute bilharzial dysentery.*—In many human cases large deposits of ova may occur in the intestine without any attempt at papilloma-formation, and these are the cases which in life suffer from bilharzial dysentery. There is a generalised diffuse thickening of the mucosa and submucosa with acute inflammation but without ulceration, and, as in the monkey intestine, there is the same hypersecretion of mucus by the goblet cells.

3. *Adeno-papillomata of the colon.*—These arise, as has been described, from the submucosa, and their structure and general appearance are so well known that they do not call for further description or comment here.

4. *The Pathology of other Abdominal Structures.*

In artificially infected monkeys, ova of both varieties of bilharzia are commonly found in the venules of the small intestine; generally, however, any associated pathological changes are confined to the ileum.

Microscopical Changes.—Though actual papilloma-formation has never been observed, yet signs of bilharzial inflammation—infection of capillaries, denudation of surface epithelium, increased mucoid secretion and petechial hæmorrhages are all present. Subserous tubercles may be noted in the last two or three feet of the ileum. The cellular exudate in the lumen of the gut resembles that of the large intestine and is composed for the most part of polymorph cells—in contrast to the eosinophilia found in the pulmonary alveoli and in the bladder.

Secondary bacterial infection of the sinuous tracts opened up by the exit of ova through the submucosa and mucosa during their passage into the intestinal canal would appear to be responsible for this.

The subserosa shows a subacute patchy inflammation with occasional hæmorrhages and egg abscesses.

The muscular coats are, as a rule, unchanged.

In the submucosa the same changes are found as in the large intestine and adult parasites are present in the veins and in the subperitoneal tissue, especially at the mesenteric attachment.

Stomach and duodenum.—In *B. mansoni* infections in monkeys occasional ova have been noted in the venules, but in one case only macroscopic lesions, *i.e.* subserous pseudo-tubercles, have been noted. I understand that in man lesions are also exceptionally rare. Microscopic sections have demonstrated lesions of the duodenum similar in nature to those described as occurring in the ileum, with, in addition, involvement of Brunner's glands.

Pancreas.—By digesting this tissue with caustic potash both lateral and terminal spined ova have been seen; macroscopic lesions of the organ have been demonstrated in one case only, namely, in a monkey heavily infected with *B. mansoni*. The microscopical changes in this case were very characteristic. The lesions had a definite anatomical distribution, and consisted of an eosinophil infiltration of the perilobar fibrous tissue in which ova and giant cell systems occurred.

Pigment was noted in certain endothelial mononuclear cells and streams of eosinophils infiltrating the lobular zone and isolating groups of glandular cells were present. Cell islets were common in the sections. Such lesions must inevitably culminate in the production of a chronic interstitial pancreatitis (perilobar).

Spleen.—Macroscopically the spleen is enlarged and congested and on section the Malpighian corpuscles are prominent.

Microscopically the following changes have been noted:—

1. Acute venous congestion, which may be so extreme as to cause secondary atrophy of the pulp.

2. Proliferative activity of the large mononuclear cells in the centre of the Malpighian corpuscles with evidences in extreme cases of focal necrosis, such as pyknosis and nuclear fragmentation.

3. The presence of numbers of macrophage cells bearing pigment—probably a derivative of hæmoglobin and an index of the amount of hæmolysis; these are generally found in the centre of the lymph nodes. Sometimes, however, pigment is found scattered throughout the whole pulp.

Neither macroscopic nor microscopic bilharzial cell accumulations have ever been observed; that they must occur is shown by the fact that occasionally ova may be demonstrated in splenic pulp digested with 3 per cent. caustic soda.

The early implication of the spleen in experimentally infected monkeys is analogous to the painful enlargement of this organ, which is a prominent clinical feature in man during the second and third month after infection in acute bilharziasis, and is probably similar to that described in katayama disease. The underlying pathological basis is probably identical, and is due, not to the deposition of ova, but to the absorption of some circulating toxin.

Focal lesions of the spleen and pancreas in man appear to be as rare as they are in experimentally infected monkeys. Professor Ferguson informs me that he has never observed focal lesions of the spleen amongst the Egyptian fellaheen. Symmers (1906 [10]) once described an interstitial pancreatitis associated with ova in a case of diffuse bilharziasis in man. Probably the escape of these organs is due to the fact previously emphasised—that the adult bilharzial parasites appear to rarely inhabit the splenic vein.

Lymphatic glands.—The lymphatic glands of the mesentery and mesocolon have been particularly studied. To the naked eye they appear markedly softened and enlarged. On digestion of the tissues ova are always found.

MICROSCOPICALLY, the picture resembles an irritative lymphadenitis. Quite apart from the existence of egg foci in the gland pulp beneath the cortical sinus, there is a generalised congestion. The lymph nodes are highly cellular and their centres show a definite proliferative activity of the large mononucleated elements. The subcortical sinus system is, in many places, crowded with eosinophil cells, as is also the central lymph sinus system. It is noteworthy that the eosinophils in the latter system present a much fresher appearance than those in the periphery of the gland, and also that the infiltration of the gland pulp with eosinophil cells is quite generalised.

In *human* subjects a comparable series of changes may be observed at a later date in the mesenteric and mesocolonic glands in cases of diffuse bilharziasis of the abdominal viscera. The invaded gland may contain so many egg foci with their associated histological changes that the picture of acute tubercle is simulated.

Microscopically the common cell is of the small mononucleated variety.

5. *The Urinary Bladder and Uterus in Experimental Bilharziasis.*

The extent to which the bladder is involved in the early stages of infection with *B. hæmatobia* is one of the most striking features of the pathology of bilharziasis.

Within the first three months of exposure to infection, the walls are thickened to such an extent with bilharzial deposits and the mucous surface so beset with papillomatous growths that its capacity is greatly diminished. Each individual papilloma may vary in size from 1–10 mm. and may be pedunculated or arise from a broad base. Their coloration, when fresh, often bears a bluish tinge which spreads to the mucous membrane in the vicinity. In extreme cases the

lumina of the lower third of the ureters may show dilatation and their walls considerable thickening. Dilatation results from obstruction and thickening from the cellular infiltration of the walls in the vicinity of deposited ova.

In monkeys obstruction of the ureteric orifice has never been sufficiently complete to produce hydronephrosis, which is a common sequela of this condition in man.

Bladder lesions are due almost exclusively to *B. hæmatobia.* In only one case of *B. mansoni* infection were any microscopic lesions of the bladder visible, and consisted of a few small pseudo-tubercles scattered throughout the subperitoneal tissue and the submucosa, but there was no attempt at papillomatous formation.

Microscopically the coats of the bladder show the following structural changes :—

The serous coat is œdematous and the seat of hæmorrhage and cell infiltration in the vicinity of the adult parasites or their ova. In the *muscular coat* individual fibres are œdematous and there is an infiltration of the intermuscular connective tissue with eosinophils. The adult parasites, especially the females, may be met with in the veins situated in contact with the serosa, in the middle muscular layer or in the *tunica propria* itself. As in other tissues, the same intense grade of eosinophilia, especially around the adult parasites and their ova, can be observed.

In the *mucous coat* the gradual evolution of the adeno-papillomata can be studied. At first the free surface of the mucosa becomes corrugated with a number of minute elevations, formed for the most part of eosinophil cell accumulations, the result partly of irritation by the ova and partly by toxic action (Plate XI. Fig. 4*a*).

As soon as these papillary outgrowths have attained any appreciable size, a corresponding downgrowth of the epithelium in columns takes place through the aggregated cells, to such an extent as, at first sight, to suggest malignant disease. There is, however, no cellular infiltration of the lymphatics and the histological continuity of the downgrowths with the surface epithelium can be traced with ease.

Although actual malignancy does not occur in early papilloma-formation, it is, nevertheless, a very real danger at a later period, in long-standing vesical bilharziasis, as Professor Ferguson (1911 [11]) has so ably demonstrated. The real significance of these cellular downgrowths should not be lost sight of.

It would appear, then, that the vesical papilloma is due to two factors—

(*a*) The irritative action of toxins emanating from the ova, provoking hyperplasia of both surface and glandular epithelium.

(*b*) The mechanical forcing upwards of the mucous membrane by cell accumulations in the lower strata of the bladder wall.

In most papillomata, terminal spined ova are present, but not necessarily in large numbers; it would appear that the tissue changes concerned in the production of papillomata take place without any direct relation to the number of ova deposited; indeed, histological studies would suggest that their size and formation depend much more upon the nature of the leucocyte response and the extent of the proliferation of the surface epithelium.

The uterus.—In female monkeys, heavily infected with *B. hæmatobia,* pseudo-tubercles have been noted in the submucous and serous coats; in *B. mansoni* infections no such lesions have been observed.

In the endometrium typical bilharzia cell accumulations of the usual type were present, that is, eosinophils, giant and mononuclear cells around the characteristic ova (Plate XI. Fig. 3). Some had undergone central degeneration. The muscular fibres were in places œdematous, and were infiltrated with eosinophils. On section, adult parasites were found in the vessels of the subserosa.

Corresponding lesions at such an early stage of the disease have never been observed in human beings, but the more chronic conditions, in which many ova were found to be calcified and the walls of the uterus to be thickened by a considerable deposit of fibrous tissue, are commonly met with in Egypt.

6. *The Lung in Experimentally Infected Monkeys.*

The lung may be extensively involved, especially in the early stages of *B. hæmatobia* infection. Pulmonary lesions were only met with in 10 per cent. of experimental infections with *B. mansoni*, and are never so widespread as in the former.

Macroscopically the lesions closely resemble those of miliary tuberculosis, and, indeed, in very heavy infections they may be spread uniformly throughout all the lobes of the lung. In their microscopic structure, too, there is a considerable resemblance.

The same cell picture as described elsewhere is present, and in the larger nodules ova can invariably be demonstrated in section. In the intermediate neighbourhood of these nodules the pulmonary alveoli show proliferation of the endothelium of a subacute inflammatory nature.

Broncho-pneumonia. — In certain portions of the lung definite solid patches of broncho-pneumonia can be found, in the centre of which, recently defunct, and it may be immature, bilharzia parasites surrounded by extensive cell infiltration can be demonstrated. The parasites are the central point of these solid patches, and one must remember that they are invariably situated in the centre of a blood vessel in which they appear to have become impacted; and that the nodular mass is composed, for the most part, of eosinophil cells in a serous coagulum which have collected round to such a degree, that in hæmatoxylin sections, counterstained by eosin, these patches appear bright crimson in colour (Plate XI. Fig. 4*b*). The pulmonary alveoli in the neighbourhood are also full of eosinophil cells and sero-fibrinous exudate. The vessels in which the parasites are encountered display great attenuation of the muscular coat, but no compensatory proliferation of the endothelium or any perivascular cell exudation. Intra-alveolar hæmorrhages are uncommon despite the considerable arterial obstruction caused by plugging by the adult parasites, but there is evidence that a certain amount of lymphatic obstruction may occur, for perivascular lymphatics packed with lymphocytes may be seen radiating from the larger broncho-pneumonic patches. The bronchi and bronchioles are, generally speaking, free from any inflammatory disturbances.

Pigment.— In the lung tissues the golden-brown bilharzial pigment is found within the bodies of the adults as well as in the vicinity of the ova. It can be demonstrated within the pulmonary alveolar walls for considerable distances from the location of the parasite itself. In arterioles which have become completely obstructed by dead parasites the same pigment may be demonstrated within the arterial muscular coats, whither it has been transported from the intestinal cæca of the worm by the action of phagocytes.

Pulmonary bilharziasis in man.—In the limited number of cases of acute bilharziasis recently observed in man, there have been pul-

monary signs and symptoms which suggest that they have a pathological basis similar to the one I have described above as occurring in monkeys. It is known that in prolonged infection in man these early changes culminate in an interstitial pneumonia focused around egg deposits and long-defunct parasites. In a few cases in which I have been able to study pulmonary lesions in man, these were accompanied by a deposition of ova and the typical cell exudate. Such lesions, however, never were as extensive as those produced in hyperinfected monkeys.

The frequency with which the lungs are involved in pure *B. hæmatobia* infections can be gathered from Turner's (1909[12]) figures from South Africa—namely, that 93 out of 131 cases, or 70·9 per cent., showed deposits of ova in the lungs.

7. *The Kidney in Experimental Bilharziasis.*

Bilharzial lesions of a size visible to the naked eye have never been met with in either type of infection (*B. hæmatobia* or *B. mansoni*).

At autopsy, as a rule, the kidneys are considerably swollen and congested. Some of the following microscopical changes are observed:—The cells of the convoluted tubules of the cortex are undergoing a granular degeneration and their lumen is packed with a similar kind of granular material. The glomeruli are swollen with polymorph and eosinophil cells; both afferent and efferent capillaries are in a similar condition; on the other hand, there is no serous exudation into the capsular space. Scanty ova may be demonstrated by digesting the whole organ with 3 per cent. caustic soda; they are generally in the intertubular connective tissue and are surrounded by the typical cell exudate. While the focal lesions of bilharziasis in the kidney resemble those in other portions of the body, yet the degeneration of the renal tubules and the congestion of the vessels are indicative of a generalised bilharzial toxæmia.

In man, although secondary septic involvement is a common terminal complication of vesical bilharziasis, gross primary implication of the kidney appears to be a rare event.

VI.

THE CELLULO-HUMORAL RESPONSE IN EXPERIMENTALLY INFECTED MONKEYS.

(A) Observations on the Blood and Bone-marrow in Monkeys spontaneously recovering from the Disease.

An analysis of Table I. shows that in monkeys recovering from the initial toxic effects of a severe infection, a remarkable cellulo-humoral response is evoked, thus:—

1. A strongly positive complement fixation reaction[1] developed in every case.

[1] I have described elsewhere in detail (*R.A.M.C. Journ.*, 1919[2], vol. xxxii. p. 449) the technique of this reaction. The antigen is prepared from an alcoholic extract of the digestive gland (or liver) of snails (*Planorbis boissyi*) infected with the cercariæ of *B. mansoni*.

2. A leucocytosis was always present, varying from 13,000 to 34,000 leucocytes per c.mm. during the fifth to the twelfth weeks of the disease.
3. Within the same period the eosinophilia varied from 10 per cent. to 63·2 per cent., and the eosinophil count averaged 28 per cent.

In the heavier infections some grade of secondary anæmia was observed; this was never so severe, however, as in those animals which early succumbed to the disease.

Blood culture in monkeys during the first three months of the disease was always sterile. The blood was obtained from the heart by intraventricular aspiration. There would therefore appear to be little grounds for Archibald's (1914 [13]) suggestion that the febrile symptoms of intestinal bilharziasis are due to a secondary bacterial infection of the bowel wall.

The *bone-marrow* in these cases was in a state of general activity, and presented evidence of both a leucoblastic and an erythroblastic response (Plate XIII. Fig. 6). While signs of cell degeneration are absent, there are many evidences of nuclear activity, division occurring both by direct fission and by mitosis. There was a definite increase in the eosinophil elements, both in the myelocyte, the polymorpho-nuclear, and all intermediate stages. There was no corresponding degree of activity noticeable in the neutrophil polymorpho-nuclear cells. Regarding the erythrogenic elements, normoblasts in varying stages of nuclear division occur abundantly.

Table I.

THE IMMUNITY RESPONSE IN MONKEYS RECOVERING FROM ACUTE BILHARZIASIS.

No.	Species.	Time of Observation.	Complement Fixation Test.	Percentage of Eosinophilia.	Leucocytes per c.mm.	Autopsy.
1	*B. mansoni*	11th week	Positive	22·2	15,000	Lateral spined ova and characteristic worms.
2	,,	5th ,,	,,	21·6	13,000	,,
3	,,	7th ,,	,,	10·7	17,500	,,
4	,,	8th ,,	,,	63·2	28,000	,,
5	,,	7th ,,	,,	24·0	14,700	,,
6	,,	8th ,,	,,	41·4	34,400	,,
7	,,	6th ,,	,,	35·0	27,000	,,
8	,,	12th ,,	,,	30·8	...	,,
9	,,	12th ,,	,,	15·5	13,800	,,
10	*B. hæmatobia*	9th ,,	,,	16·6	18,000	Terminal spined ova and characteristic worms.

(B) Observations on the Blood of Monkeys dying early in the Disease.

In these monkeys the signs of severe secondary anæmia, such as diminished hæmoglobin and red blood corpuscles, are not uncommonly observed accompanied by poikilocytosis, anisocytosis, polychromatophilia normoblasts and even occasionally by megaloblasts.

The real significance of these blood changes is borne out by the lack of cellulo-humoral response,—the result of mass-infection as shown in the accompanying Table II., which is so obvious as to require no further explanation.

It may be noted that monkey No. 14 died, even though the cellulo-humoral response appeared adequate. In reality, however, death was due not to toxæmia, but to acute bilharzial dysentery.

Table II.

The Cellular-Humoral Response in Monkeys dying from Bilharziasis.

No.	Species of Infection.	Time of Observation	Complemen Fixation Tes	Percentage of Eosinophilia.[1]	Leucocytes per c.mm.	Autopsy.
11	*B. mansoni*	9th week	Negative	0	1,250	Lateral spine ova and charac teristic worms.
12	,,	8th ,,	,,	11·9	8,800	,,
13	,,	6th ,,	,,	18·0	18,000	,,
14	,,	7th ,,	Positive	50·2	25,000	,,
15	,,	8th ,,	,,	11·0	6,000	,,
16	*B. hæmatobia*	3rd ,,	Negative	0·6	2,200	Terminal spine ova and charac teristic worms.
17	,,	5th ,,	,,	...	2,200	...
18	,,	2nd ,,	,,	0·3	3,000	...

VII.

CONCLUSIONS.

1. The various pathological lesions produced by *B. mansoni* and *B. hæmatobia* are dependent on the different habits of the adult parasites of the two species, and not on differences in the local inflammatory or cellulo-humoral response of the tissues of the host, which are identical for both species.

[1] In the Grivet monkey the homologue of the eosinophil in man is a larger cell than the polymorpho-nuclear leucocyte and contains large oxyphil granules. The protoplasm, however, has a greater affinity for the basic stain, and therefore in staining by Leishman's method it is necessary to wash out longer than usual with distilled water in order to demonstrate the oxyphil nature of the granules.

2. The ova-producing capacity of *B. hæmatobia* appears to be much greater than that of *B. mansoni*; the maximum number of ova counted in the uterus of a female worm of the latter species being three as contrasted with fifty observed in the former.

3. The habitat of the worms of *B. mansoni* is the hepatic and portal veins, the superior and inferior vena cava. In *B. hæmatobia*, while worms also occur in these situations, the pelvic plexuses of veins are those mainly inhabited, and, as these plexuses drain into the inferior vena cava, it is not surprising that *B. hæmatobia* worms are found in the pulmonary arterioles at autopsy.

4. The distribution of both terminal and lateral spined ova in the tissues of the body is very generalised and may occur in the liver, pancreas, spleen, stomach, duodenum, small and large intestine, bladder, uterus, lung, myocardium and kidney.

In *B. mansoni* the maximum deposition of ova occurs in the liver, colon and ileum, while in *B. hæmatobia* it occurs in the bladder, uterus, lung, liver and colon.

5. In monkeys experimentally infected with *B. mansoni*, pathological lesions are most marked in the liver, colon and small intestine, while in those infected with *B. hæmatobia* they occur in the liver, colon, bladder, uterus and lung.

6. The pathological picture occurring in monkeys dying before ova are deposited in the tissues (*i.e.* within five weeks of infection) is very characteristic, and is indicative of an acute toxæmia, the brunt of which is falling on the parenchyma of the liver, the spleen and the cortical epithelium of the kidney.

7. The pathological lesions observed after the sixth week are characterised by the appearance of pseudo-tubercles in the various viscera, and perhaps by the appearance of definite papillomata. The morbid lesions here described constitute the underlying basis of the various clinical syndromes manifested in the early stages of bilharziasis in man.

8. On microscopical examination, a typical bilharzia tubercle is found to be composed of one or more centrally placed ova with adjacent giant-cell systems. Surrounding these is a dense cellular zone composed of eosinophil polymorpho-nuclear cells and small mononuclears. Degeneration of the centrally situated cells may ensue, with pyknosis of nuclear material. Identical histological lesions may be observed in man in the earlier stages of infection.

9. Special emphasis needs to be placed on the constant occurrence of hepatic involvement in both *B. hæmatobia* and *B. mansoni* infections. Bilharzia pigment occurs in Küpffer's cells in *both* species of infection, and in its physico-chemical characters resembles closely, or is identical with, malaria pigment. It possesses neither the physical nor the chemical properties of melanin.

10. Pulmonary involvement is especially characteristic of *B.*

hæmatobia infections and is due to ova, worms and their toxins being filtered out in the pulmonary arterioles,—reaching this destination from the pelvic plexuses of veins viâ the vena cava.

11. There is a definite relationship between the cellulo-humoral response in experimentally infected monkeys and the prognosis. In hyperinfected monkeys dying within five weeks, there was a constant leucopenia, absence of eosinophilia and a negative complement fixation reaction. In monkeys surviving the sixth week of infection, there was constantly present an eosinophil leucocytosis associated with a positive serological reaction.

12. The death of hyperinfected monkeys prior to the deposition of ova, and the constant presence of positive serological reactions in monkeys recovering from the initial stages of infection, go far to prove the action of some toxic body elaborated by the metabolic activities of these parasites, and of protective immunisation of the definitive host by antibody production.

I am deeply indebted to Professor A. R. Ferguson and Major P. H. Manson-Bahr, D.S.O., R.A.M.C., for assistance rendered me during the present investigation. In addition, Captain J. K. Lund, R.A.M.C., has presented me with the excellent coloured plates accompanying this article, while Major Manson-Bahr is responsible for the series of microscopic drawings. To all these gentlemen my best thanks are due.

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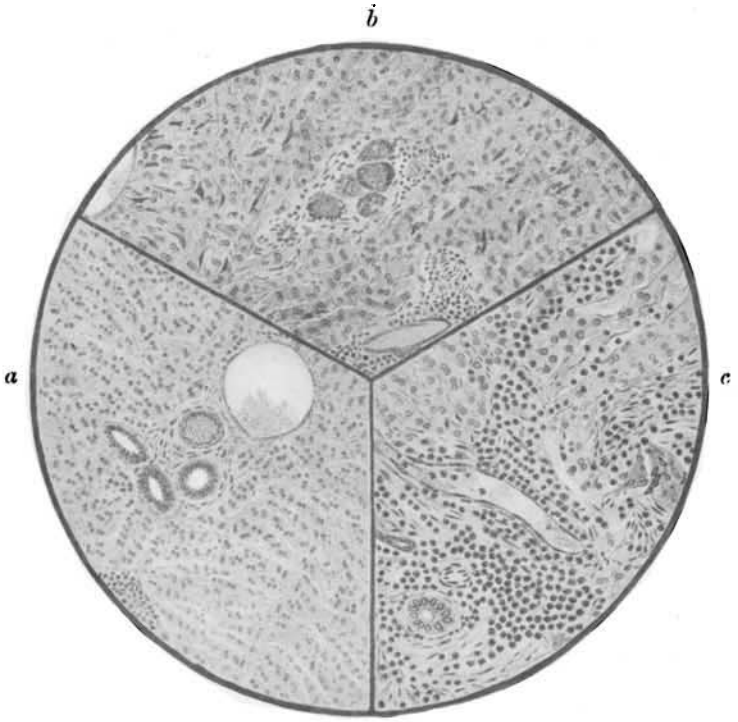


FIG. 1.

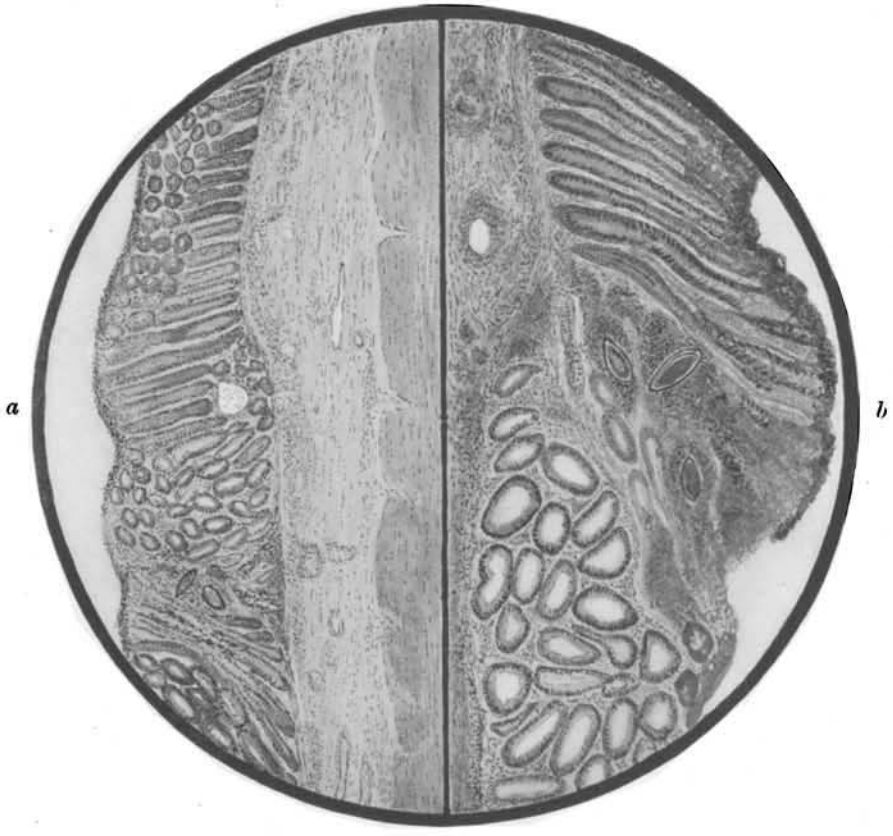


FIG. 2.

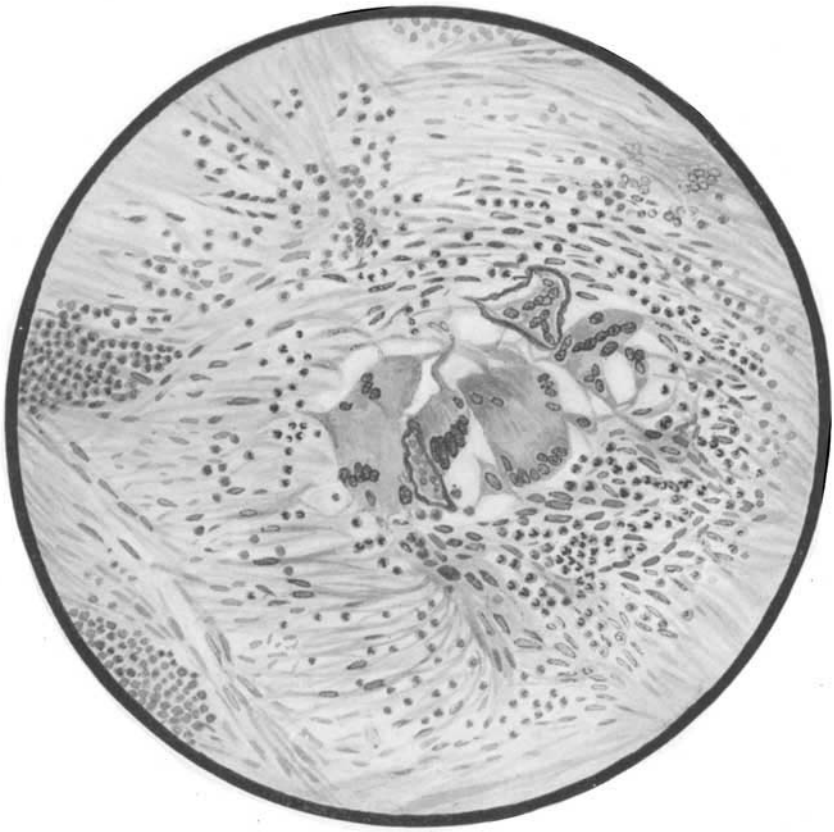


FIG. 3.

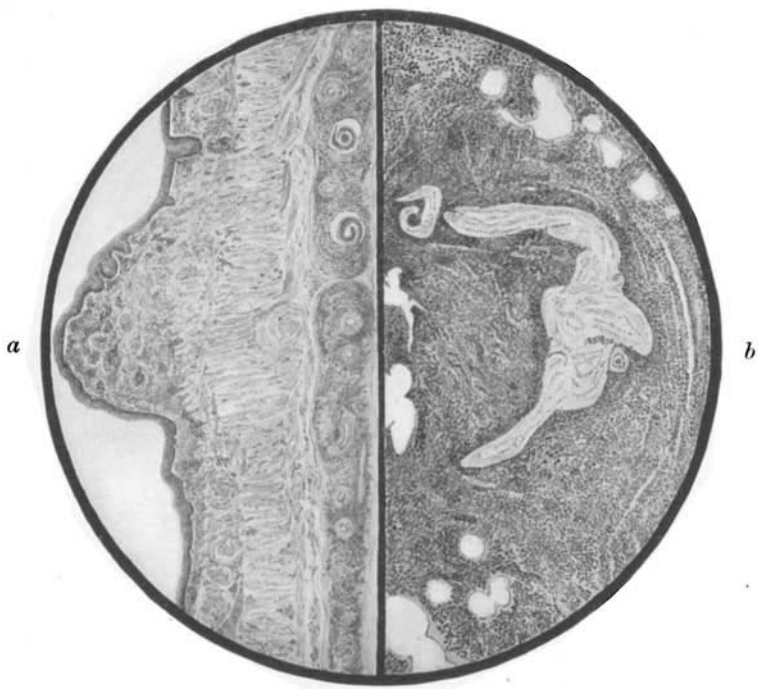


FIG. 4.

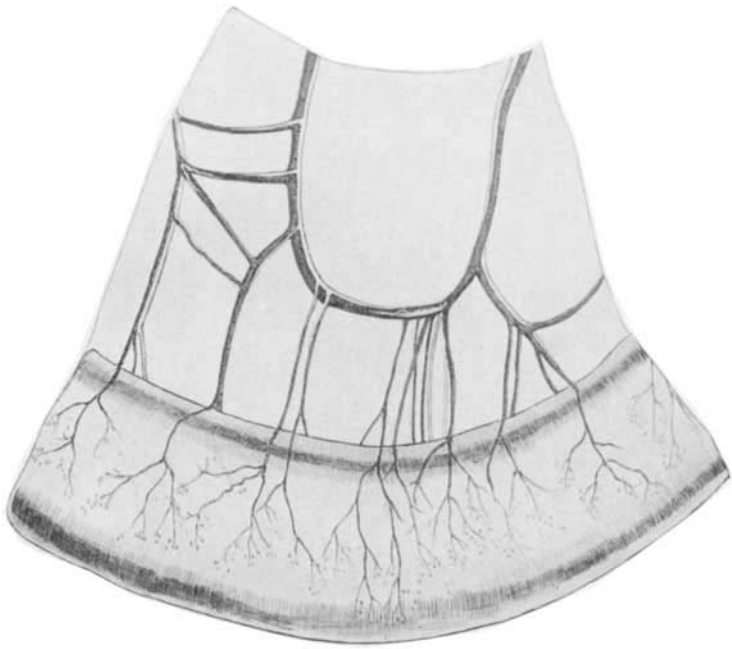
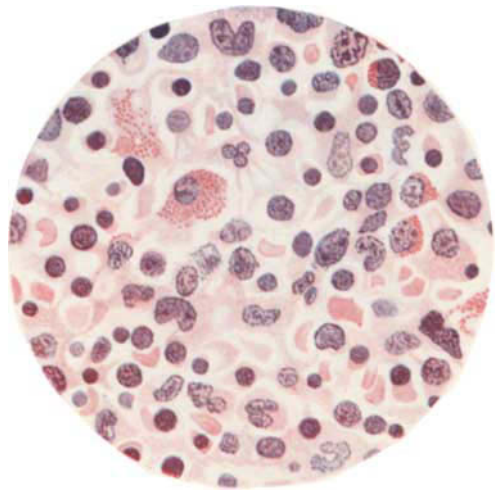


FIG. 5.



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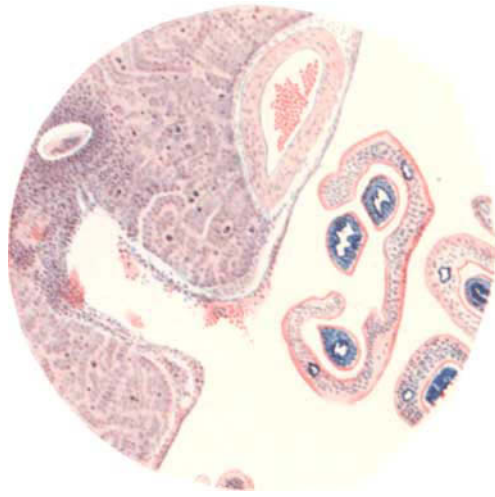

FIG. 7.

FIG 8.

FIG. 9.

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DESCRIPTION OF PLATES X.–XIV.

PLATE X.

FIG. 1*a*.—Section of monkey's liver twenty-one days after infection with *B. hæmatobia*, showing commencing periportal infiltration and toxic spoiling of hepatic cells. (×260.)

FIG. 1*b*.—Section of monkey's liver forty-two days after infection with *B. mansoni*, showing, on portion of an egg, deposition of pigment (giant cell formation and commencing fibrosis). (×260.)

FIG. 1*c*.—Section of monkey's liver sixty-two days after infection with *B. mansoni*, showing infiltration with eosinophil cells. Giant cell formation and extensive fibrosis. (×400.)

FIG. 2*a*.—Section of colon of monkey infected with *B. mansoni*, showing passage of ova through mucosa and surrounding stigmata of toxic elimination. (×70.)

FIG. 2*b*.—The same under a higher power. (×300.)

PLATE XI.

FIG. 3.—Section of uterus of monkey infected with *B. hæmatobia*, showing giant cell formation round remnants of ova; great proliferation of fibroblasts and aggregations of eosinophil cells. (×500.)

FIG. 4*a*.—Section of monkey's lung infected with *B. hæmatobia*, showing defunct immature worm surrounded by inflammatory tissue. (×200.)

FIG. 4*b*.—Section of monkey's bladder infected with *B. hæmatobia*, showing adult worms *in situ* and production of mucous papillomata.

PLATE XII.

FIG. 5.—Diagram of small intestine and mesentery of monkey, artificially infected with *B. mansoni*, showing adult worms in a loop of the mesenteric vein and the deposition of characteristic ova in the walls of the intestine.

PLATE XIII.

FIG. 6.—Preparation from bone-marrow of monkey in experimental bilharziasis, showing excess of eosinophil myelocytes. Stain Gauducheau. (× 1000.)

FIG. 7.—Section of liver of monkey experimentally infected with *B. mansoni*, showing paired worms in portal vein and ovum, with small celled infiltration. Stain Gauducheau. (× 150.)

PLATE XIV.

FIG. 8.—Specimen of colon and mesentery of monkey, showing typical pericolonic bilharzial infiltrations (*B. mansoni*).

FIG. 9.—Liver of monkey experimentally infected with *B. mansoni*, showing bilharzial pseudo-tubercles situated beneath the hepatic capsule.