THE MORPHOLOGY OF THE PARASITE (HISTOPLASMA CAPSULATUM) AND THE LESIONS OF HISTOPLASMOSIS,¹ A FATAL DISEASE OF TROPICAL AMERICA.²

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PLATES XIX-XXIII.

Soon after the occupation of the Canal Zone by Americans, in 1904, the medical officers of the Sanitary Department, on account of the numerous cases of splenomegaly met with among the Colombian natives, had their attention called by the work of Leishman³ and Donovan⁴ to a very fatal endemic disease of India, known as kala-azar. The clinical features of this disorder being splenomegaly, emaciation, cachexia, irregular pyrexia, leukopenia and the presence of an intracellular protozoon in the endothelium of the liver, spleen, lymph nodes, etc.

Manson⁵ in one of his prophetic utterances has stated that kala-azar or an analogous disease might be found in America, and before this, Ronald Ross, during his visit to Panama in 1904, looked through the wards of Ancon Hospital in search of a case of splenomegaly associated with the kala-azar symptom-group, but none was found, although tropical splenomegaly is commonly seen in this region, particularly among the native Colombians; so common, indeed, that in the autopsy room it has been the custom to speak of examples of tropical splenomegaly met with here as “Colombian Spleens.” The size of the Colombian spleen is due partly to an interstitial fibrosis and partly to lymphoid hyperplasia; etiologically,

² Received for publication April 2, 1909.
³ Leishman, W B., Brit. Med. Jour., 1903, i, 1252; 1903, ii, 1276; 1904, i, 393.
⁵ Manson, P., Lectures on Tropical Diseases, Chicago, 1905.
Morphology of Histoplasma Capsulatum.

it is not in any way related to kala-azar or to the disease described here.

During 1905–1906, while the writer, in search for the Leishman-Donovan body, was examining smears from the spleen, liver and rib-marrow of all cadavers at Ancon Hospital in which there was splenomegaly, he was rewarded by finding the microorganism associated with the lesions described here and elsewhere.

CLINICAL NOTES.

Two of the patients were Martinique negroes and the third was a Chinese who had lived on the Isthmus of Panama for 15 years. They had been ill several months and were much emaciated. All had had fever, but malarial parasites could be found in the blood of two of the patients neither before nor after death. A pronounced anemia was observed in all three cases. One patient had a leucocytic count of 2,200 per cu. mm. just before death. The most striking and characteristic feature, however, was the enlargement of the spleen. The temperature of one of the patients was observed for a period of three weeks when it was at first irregularly continuous and later regularly remittent about two degrees above normal. The irregularly continuous temperature may have been partly due to the malarial infection which was present at that time. The disorder is seen then to possess certain points of resemblance to kala-azar, viz., splenomegaly, irregular pyrexia, leukopenia, anemia, emaciation and chronicity.

Animal inoculations (guinea-pig) from material from Case III were negative and the microorganism disappeared from cultures and specimens of macerated tissue, probably on account of contaminating post-mortem bacteria which overgrew everything.

Probably on account of the vastly improved hygienic conditions in which the laborers live, this disease has disappeared for the present in the Canal Zone, but the writer believes that it will be found in other regions of Central and South America and the West Indies. It is to be noted that two of the cases were in Martinique negroes.

Cultural experiments and animal inoculations from material obtained during life from splenic and hepatic punctures will be of the greatest value in further attempts to assign a place to this microorganism in nature.
Judging from the extent of tissues invaded and destroyed by the parasite, Case III, that of the Chinese, is regarded as the advanced stage of the disease; while Case II, one of the Martiniquans, is thought to represent the earlier phase as the lesions were not so extensive and there were relatively fewer parasites, death in this case being due to an intercurrent broncho-pneumonia. Case I is intermediate in regard to the extent of tissue invaded, number of parasites and of organs attacked.

ANATOMICAL FINDINGS.

Post-mortem changes were delayed. The bodies were kept at a temperature of 74 to $92^\circ$ F., yet the decomposition usually seen in bacterial infections was absent. Emaciation was well marked, though not extreme.

Skin.—A few recent papules, 8 mm. in diameter, were observed in the skin of the right arm in Case III.

Subcutaneous Tissue.—Slightly edematous in Case III.

Musculature.—Atrophy of all the muscles, particularly the sartorius muscle in Case III.

Pleurae.—There were a great many ecchymoses, 8 mm. in diameter, scattered beneath the visceral pleura of the lungs in Case I, which corresponded to underlying hyaline nodules, 2 to 4 mm. in diameter. Fewer of these hemorrhages were seen in Case III and they corresponded to the smaller number of pseudo-granulomata noted in this case.

Lungs.—In Case I, the lungs presented a picture very much like that of miliary tuberculosis, for on section the lungs were dotted everywhere with dense pale gray hyaline granulomata, 2 to 3 mm. in diameter. The granulomata were not so numerous or so closely packed as in miliary tuberculosis and they were surrounded by narrow margins of hemorrhage. In Case III the granulomata were in some instances elongated and larger, as though several smaller ones had coalesced. These too were surrounded by an area of hemorrhage. These granulomata were always of a peculiar grayish hyaline tint and were quite dense and smooth on section. No granulomata were detected in the lungs in Case II, which showed the mildest or lightest infection, but there was a terminal broncho-pneumonia.

Peribronchial Lymphnodes.—These were enlarged and contained black pigment in all cases, and in Case I there were a few small fibro-caseous tubercles.

Heart.—This organ was small in all cases and was proportionate to the atrophy of the other muscles.

Liver.—In the advanced stage of the disease as shown in Case III, the liver presented a very unusual appearance, due to extensive necrosis and cirrhosis. The organ was enlarged and its capsule smooth, while scattered throughout there were many areas of necrosis, grayish yellow in color, the larger ones...
frequently being arborescent in shape. The arborescent areas involved the portal radicals which they followed; their centers were gray and their peripheries yellow. The arborescent twigs had four to six limbs. They were not so numerous near the falciform ligament as elsewhere.

In the earlier stage of the disease as represented by Case II, the necroses were much smaller, fewer in number and were not visible macroscopically.

**Portal Lymphnodes.**—These nodes in Case III were enlarged, pale yellow, slightly edematous and friable.

**Spleen.**—The spleen was very markedly enlarged in each case, varying from about 450 to 750 grams in weight. It was more suggestive of the appearance and consistence of the organ in spleno-myelogenous leukemia than of anything else and was distinctly different from any other type of spleen met with in Panama. The capsule was smooth and tense. The entire organ was rigid and not flexuous to the slightest degree. The pulp was firm in consistence yet friable. The pulp was dark red in color and the Malpighian corpuscles were visible in Case I and indistinct in Case III.

**The Lymphnodes at the Hilum of the Spleen.**—These were enlarged and pale.

**Pancreas, Kidneys and Adrenals.**—There was nothing of note.

**Intestines.**—Case III presented a number of very curious areas of pigmentary, hyperplasia and ulceration in the jejunum, ileum and colon; there were about fifty in the former and twenty-four in the colon. The earliest lesion appeared to be that of hyperplasia or the formation of a small nodule in the mucous and submucous coats; these nodules were 5 to 6 mm. in diameter, and later, became reddened and infiltrated around their peripheries; necrosis and circular ulceration appeared, sometimes with hemorrhage from the ulcer. The last phase was that of a puckered, pigmented, black scar about 6 or 8 mm. in diameter. The size, distribution and peculiarly black pigmentation of these ulcers with the black pigmentation of the neighboring visceral and parietal peritoneum made them of striking appearance and unusual interest. The posterior lymphnodes were enlarged and pale, while the mesenteric lymphnodes in this case were not appreciably enlarged.

In Case I there were a few superficial circular ulcers in the cecum and ileum, and some hyperplasia of the mesenteric lymphnodes. The bone-marrow, brain and membranes, accessory nasal sinuses, joints, blood vessels and genitalia, showed no gross lesions referable to the infection.

**THE PARASITE.**

The microorganism was present in enormous numbers in the tissues, generally intracellular in large mononuclear endothelial cells in the liver, spleen, lymph nodes, submucous nodules in the ileum and colon, and in the hyaline nodules in the lungs.

Smears from the lung nodules contained great numbers of large cells resembling alveolar epithelial cells enclosing from twelve to three hundred parasites (Fig. 3).

Smears from the liver and spleen showed almost the same picture
Samuel T. Darling.

(Fig. 9). The individual cell infection was not so great; that is, there were not so many parasites in a cell but there was a very large number of free parasites.

The parasite is best seen in very thin smears from invaded tissue such as spleen, liver and lung nodules. It is round or oval in outline and in size from 1 to 4 μ through its greatest diameter; commonly this diameter is 3 μ. The larger forms are more frequently oval.

When stained by some of the polychrome blue and eosin stains—Leishman's or Hasting's stains—the entire structure is well displayed and one is struck immediately by the various forms and positions taken by the chromatoid substance or nucleus. This substance is sometimes globular or oval and is placed at one end of the parasite, or it may be distributed half way along the periphery. There may be little dot or dash-shaped off-shoots into the interior from an irregular mass or ring of chromatin at one end of a parasite. Hardly any two parasites look alike. Besides the chromatoid substance there is some blue staining basophilic substance making up the remainder of the parasite and filling out the oval or round outline, except for an achromatic zone generally placed near the center (Fig. 3).

The parasite is surrounded by a refractile capsule, one-sixth of the diameter of the parasite in width, which ordinarily does not stain. In hematoxylin stained smears it is possible, occasionally, to demonstrate a single minute dot in this refractile capsule. A number of the parasites contain one or more small chromatoid granules located in the center or on the periphery. While these chromatoid granules are generally solitary, at times two or more may be counted. A few large parasites have several chromatoid dots. These granules are frequently situated in an achromatic zone or upon the margin either near or within the basophilic substance or even in the refractile capsule.

The chromatoid granules are generally spherical, very rarely elongated, and occasionally two granules in apposition simulate a rod.

The achromatic zones resemble the achromatic zones seen in malarial rings, filaria embryos, trypanosomes and various protozoa.

The parasite appeared to divide by fission into two equal or unequal elements (Fig. 2). One apparently was dividing into four equal elements, while several parasites with chromatoid dots
scattered through their substance appeared as presegmenting bodies ready to divide into five or six elements (Case I). Occasionally a smaller parasite may be seen close beside a larger one as though separating from it, the smaller one being about 1 μ in diameter.

The lack of uniformity to the disposition of the chromatic and achromatic substance in the parasite gives a very varied picture, depending partly on the part presented to the observer.

In Case I this was particularly noticeable in smears from the lung nodule. Forms suggesting the appearance of familiar objects, such as a conch shell, bullet, shuttle, the eye and palpebral fissure were seen. The arrangement of the chromatoid substance along the periphery of the parasite in certain specimens was strikingly imitative of a mammalian embryo in "fetal attitude."

Three flagellated forms were seen in a smear from the lung in Case I, and two from a spleen smear in Case III. The distal extremity of the flagella contained a rod of chromatin placed at right angles to the flagellum, simulating the relation of centrosome to chromatin filament in *Trypanosoma lewisi*. The flagella were single, short and thick, without chromatin filaments and were enclosed by the refractile capsule of the body of the parasite.

In smears from the lungs the parasite was almost always intracellular (Fig. 3). The cells enclosing the parasite were large mononuclear cells resembling large endothelial or alveolar epithelial cells. However, as none of the invaded cells in lung smears contained dust particles they may have all been endothelial cells. The invaded cells contained from eight or ten to one hundred or more parasites of slightly varying sizes or with a larger parasite surrounded by a clump of smaller ones. One unbroken cell in a lung smear contained more than three hundred parasites which had invaded the nucleus as well as the cytoplasm. This infected cell occupied one-third the diameter of the one-twelfth oil immersion field. Smears from the spleen and rib-marrow contained fewer parasites, although still numerous, and they were often extracellular. The nucleus of the cell appeared now and then to have been invaded. Wherever found the parasite was always enclosed in a refractile capsule. Red blood corpuscles were never invaded.

One of the most striking characteristics of the parasite is the lack
of uniformity to the distribution and arrangement of the chromatic and achromatic substance.

The parasite always takes polychrome blue stains like a living or recent microorganism.

Twenty-four hours after the death of the host, the chromatin and basophilic substances stain beautifully, while under similar conditions the malarial parasite would not stain so well and the chromatin dot might not stain at all.

In rib-marrow smears it was noticed that the microorganism required more prolonged contact with the stain to obtain good results. This was thought to be due to the impenetrability of the refractile capsule.

**HISTOLOGICAL EXAMINATION.**

Tissue was fixed in Zenker’s solution and in formol, and paraffine sections were stained by eosin and hematoxylin, eosin and methylene blue, by Marchi’s, Van Giesen’s, and by Weigert’s fibrin method. A few celloidin sections were stained by eosin and a polychrome methylene blue, differentiated with anilin oil. This proved to be an excellent stain for the parasite in tissue. Gentian violet also stained the parasite intensely.

In the description of the microscopic appearance of the tissue each case will be considered separately.

Taking up Case II first, in which, while there was a considerable degree of splenomegaly, the lesions in the liver were not at all advanced and the case altogether may be regarded as an early stage of the disease; this will be followed by a description of the lesions in Case I, in which the disease was well advanced in the lungs, liver and spleen. Finally Case III, which represents a very advanced stage of the disease with the splenomegaly, extensive liver necroses, intestinal ulceration and pulmonary pseudo-granulomata will be described.

**CASE II. G. B. HOSPITAL NUMBER 9141.**

*Liver.*—The columns of liver cells everywhere appear flattened and attenuated and the capillaries correspondingly increased in diameters. Here and there are areas of necrosis, in size up to 0.2
mm. in diameter, scattered throughout the lobule, apparently ar-
ranged irrespective of zone. Some of these areas of necrosis
extended outward from portal spaces; others were in the interme-
diate zone, while others could not be oriented on account of the
obscuration of the zone limits. In these areas of necrosis the debris
has been almost completely removed, but here and there may be seen
a small patch of coagulative necrosis or granular debris, yet on the
whole the necrotic area has been replaced by young connective tissue
cells and also by exudative cells similar to those seen in the kidney.
These areas frequently contain one or more very large cells, 24 to
30 µ in diameter, distended by parasites. One of these areas of
necrosis contained a typical giant cell with a mural arrangement of
its nuclei on one side. The giant cell was not a part of a tubercle
and around it were no round cells, epithelioid cells nor nuclear frag-
mentation. This giant cell was in the intermediate zone of a lobule.
Following along the capillaries there are seen collections of parasites
in endothelial cells. These endothelial cells have oval eccentric
nuclei and their cytoplasm is invaded to a greater or less extent by
parasites. The parasites are enclosed in a colorless chamber which
in the lightly infected cells is generally much larger than that neces-
sary to enclose them, while in the more heavily infected endothelial
cells, this colorless chamber is obscured by the great numbers of con-
tained parasites. The periphery of the infected endothelial cell is
almost fibrous in character and remains intact when the nucleus and
cell contents have disappeared. Many of these infected endothelial
cells contain malarial pigment as well. The malarial pigment, how-
ever, appears to be near the periphery of the cell and not mixed in
with the parasites. This proves the endothelial nature of the para-
site-bearing cell. From an examination of this section, the earliest
lesion is apparently that of the invasion of the endothelial cell.
The portal veins in some of the medium sized portal spaces contain
a good deal of debris, also a few endothelial cells containing para-
sites and malarial pigment, or, occasionally, a leucocyte or red blood
cell. In the more lightly infected endothelial cells, the parasites
are not always of the same size; for instance, there may be one
large oval parasite and clustered around it six or seven parasites of
decreasing size. No free parasites could be detected in the section.
The capillaries contain, besides, red blood cells staining variously with eosin, a number of which are washed out and their hemoglobin scattered here and there in coarse granules.

*Spleen.*—The capsule is 80 μ in thickness and is not appreciably thickened. There is slight atrophy of the Malpighian corpuscles. The splenic spaces of the pulp are distended by red blood cells and mononuclear cells and a few polymorphonuclear leucocytes. Scattered here and there are large mononuclear cells with small round or oval nuclei, the cytoplasm being distended by parasites. Some of these lie free in the distended splenic spaces, while others are attached to the wall of the splenic space and are endothelial in character. A number of them contain malarial pigment and red blood cells, and a few contain parasites, red blood cells and malarial pigment. There is on an average two or three infected cells to the oil immersion field. Here and there are a few parasites in groups of five or ten apparently free, but by far the greatest number are intracellular. The infected cells contain anywhere from two or three to fifty or more parasites. The connective tissue reticulum does not appear to be thickened and the size of the spleen is due to the engorgement of the splenic spaces and the invasion of the endothelial cells by parasites.

*Lung.*—The capillaries are tortuous and engorged with red blood cells. The alveoli contain red blood cells and polymorphonuclear leucocytes in about equal numbers and a few desquamated alveolar epithelial cells. Quite a number of the alveoli contain coagulated albumin and a few contain some small clumps staining like fibrin. The lumen of the small bronchi contain red blood cells, polymorphonuclear leucocytes and a few desquamated alveolar epithelial cells. No parasites could be detected.

*Portal Lymph Node.*—The node shows a diffuse hyperplasia of small and large lymphoid cells, particularly the latter. The connective tissue of the germinal centers in the follicles of the cortex shows extensive hyaline degeneration. The lymph sinuses in the cortex and medulla contain great numbers of large mononuclear cells about 20 μ in diameter, with round, oval, or horseshoe nuclei 4 to 7 μ in diameter. These large cells show frequently double nuclei and mitoses and are undoubtedly being reproduced rapidly. They are
Morphology of Histoplasma Capsulatum.

ravenously phagocytic and have engulfed malarial pigment, red blood cells and a very few of them contain colorless chambers enclosing parasites, while a few others have picked up two or three parasites, as yet without the development of the achromatic chamber. The structure of the node is loose rather than dense and the small lymphocytes, which make up the bulk of the cellular elements in the dense lymphoid tissue, have deeply staining nuclei and are from 7 to 9 μ in diameter, the nucleus being from 4 to 7 μ in diameter.

Kidney.—The most striking feature is the increase in the intertubular connective tissue; both in the cortex and medulla, the tubules are separated by collections of cells, most of them of the plasma cell or polyblast type, round or oval in outline with eosin staining cytoplasm and rather large, generally eccentric nuclei. Some of them have double nuclei and a few show mitoses. In addition to these cells, which very strongly impress one as exudative, there are quite a number of polymorphonuclear leucocytes in the same localities and, occasionally, in the lumen of the convoluted tubules. Here and there are small hemorrhages into the intertubular connective tissue. The convoluted tubules are, generally, dilated and their epithelium, subcuboidal and flattened. The nuclei of the cells stain well, though not deeply. There are occasional examples of diapedesis of mononuclear and polymorphonuclear leucocytes through the epithelium into the lumen. The latter contains some hyaline casts, granular detritus, a few leucocytic plugs and some macerated desquamated epithelial cells. The glomeruli are pretty tightly enclosed by their capsules and are, apparently, normal. A few convoluted tubules show colloid degeneration of their epithelium. No parasites were detected.

Adrenal, Heart, Pancreas.—No parasites could be detected and the organs present nothing of note.

CASE I. C. D. HOSPITAL NUMBER, 7715.

Liver.—The entire section is dotted over with pale areas of necrosis in size all the way up to .8 mm. in diameter; many of them are from .24 to .8 mm. in diameter. These pale areas of necrosis involve all zones of the lobule, several of them involving two or
more lobules. Most frequently they seem to extend outwards from a portal space. In these areas there is more or less complete destruction and removal of the hepatic cells, leaving a reticulum composed of the stroma of the liver in the spaces of which are the granular and fatty remains of some of the hepatic cells, together with a great number of parasites enclosed within the endothelial cells of the part. In some of the areas there has been a slight proliferation of round cells and this appeared to be oftenest seen near a portal space. In most cases the disappearance of liver cells followed upon a granular and fatty degeneration, but in a few instances the necrosis has been coagulative in character with nuclear fragmentation. The necrosis at times fades gradually into areas of normal hepatic cells between which there are half-way stages of necrosis, while at other times an area of necrosis is outlined in large part by normal hepatic cells. In the uninvaded areas the liver cells take the stain beautifully and their outlines are quite distinct. There is no inflammatory reaction to be seen anywhere, polymorphonuclear leucocytes being very scarce. Of the leucocytes in the liver capillaries, the mononuclear elements seem to predominate. The invaded endothelial cells are the same size as those seen in the spleen. Extending outward from the areas of necrosis along the portal capillaries into regions of normal liver substance there is an almost unbroken line of endothelial cells invaded by parasites.

*Spleen.*—There is some atrophy of the Malpighian corpuscles. The splenic spaces are distented by red blood cells and some large and small mononuclear cells. Quite a number of these large mononuclear cells have engulfed red blood cells. The reticulum of the splenic spaces is not thickened. Here and there are large mononuclear cells free in a sinus or attached to its wall containing one or two dozen parasites and there are a few free parasites.

*Lung.*—Section of hyaline nodule (Fig. 4): the nodule appears to be made up of the collapsed and proliferated alveolar walls, although the capillaries of the alveoli can be made out with distinctness only near the periphery. The nodule is reticulated and scattered through the reticulum are round, oval and polygonal cells from 6 to 15 μ in diameter, their nuclei being from 4 to 5 μ in diameter. These cells are probably exudative in origin and re-
Morphology of Histoplasma Capsulatum.

semble the cells described in the intertubular connective tissue spaces in the kidney of Case II. Side by side with these cells are a few red blood cells and the much larger cells resembling endothelial cells or alveolar epithelial cells with the achromatic chambers containing parasites. While most of the parasites are intracellular, a few are free; polymorphonuclear leucocytes are conspicuously absent.

Beyond the periphery of the nodule the capillaries are tortuous and are distented by red blood cells. The alveoli are empty. The periphery of the nodule contains some alveoli enclosing red blood cells and a few large mononuclear cells with single or double nuclei containing large numbers of parasites. The parasites are sometimes within one large chamber in the cell, or there may be several smaller chambers, each one containing two or three to a dozen parasites. These mononuclear cells are from 20 to 30μ in diameter.

Lymph Node from Hilum of Spleen.—This node, particularly in the loose lymphoid tissue and in a few cortical follicles, has undergone a granular and, in places, coagulative necrosis. The dense lymphoid tissue is not so much involved but the loose lymphoid tissue everywhere shows cellular necrosis. The lymph sinuses contain large numbers of large mononuclear cells distended by parasites (Fig. 5), apparently resulting from the necrosis of the mononuclear cells containing them. Several of the cortical follicles and medullary cords show extensive necrosis and nuclear fragmentation, the central portions of which are coagulative in character. These areas contain many parasites. The lesions in this lymph node represent a more advanced stage than in those described from the lymph node in Case II.

Peribronchial Lymphnode.—This node contains several old encapsulated fibro-caseous tubercles and one giant cell. The reticulum and capsule of the node were greatly thickened in places. A lymph vessel beneath the capsule contained mononuclear cells invaded by parasites.

CASE III. Fu. Mas. Hospital Number, 17184.

Liver.—The liver presents an extraordinary appearance on account of the great destruction of hepatic cells and the invasion by parasites. There are large areas of necrosis .8 to 1.2 mm. in
diameter, irregular in outline, with extensions into the more nearly normal liver substance. The oldest areas appear to be those in and extending outward from the portal spaces, in fact every portal space is involved in an area of necrosis or its partial replacement by connective tissue and an invasion by parasites, while on the other hand several central and sub-lobular veins are surrounded by normal liver cells, but the process of destruction is so extensive that every zone sooner or later becomes involved. Fully two-thirds of the section is represented by areas of necrosis and the partial replacement by connective tissue and parasites. The destructive process in part consists of a granular and fatty degeneration of the hepatic cells and in part of a reticulated coagulated necrosis always associated by the presence of myriads of clustered groups of parasites (Fig. 8). As the hepatic cells are destroyed their places are taken by collections of parasites, which are sometimes free, but oftener enclosed in large mononuclear cells representing the endothelial cells of the capillaries. These enormously distended endothelial cells so fill up the spaces formerly occupied by the hepatic cells that there is no gross contraction or distortion of the framework (Fig. 7). In the older areas of necrosis the connective tissue stroma had become fibrous with relatively few cells, and the lymph spaces of this reticulum are distended by intracellular and extracellular collections of parasites. In the areas of coagulative necrosis there are numerous free parasites, either singly, or in twos, or threes. Throughout the section there are large patches involving portions of three or four lobules in which the hepatic cells are flattened and atrophied. The hepatic cells here contain much bile pigment and small fat droplets. The capillaries are engorged with red blood cells and a number of the endothelial cells are distended by parasites. One of the sub-lobular veins which is surrounded by an area of necrosis contains a large cell attached to its wall projecting out into the lumen. This cell is 24 by 27 μ in area, its nucleus is oval 4½ by 6 μ in area and is eccentric. This cell contains a clear central chamber 25 μ in diameter filled with parasites of varying sizes. This cell is almost free from its attachment, three-fourths of its periphery bulging out into the lumen.

The heaviest invasion by parasites was in the connective tissue
Morphology of Histoplasma Capsulatum.

and endothelial cells, which had replaced the areas of necrosis; these areas correspond to the hyaline arborescence noted at autopsy.

The capsule of the liver was thickened and frequently communicated with the infected underlying areas of necrosis, but the capsule showed no invasion by parasites. The epithelium of the bile capillaries was not invaded by parasites.

Spleen.—The capsule is uniformly 0.06 μ in thickness. The Malphigian corpuscles show considerable atrophy of their lymphoid elements and there is an increase of fibrous tissue around the artery and in the stroma of the corpuscle. The splenic spaces are greatly distended by red blood cells. The connective tissue framework of the spleen is not thickened and the great bulk of the spleen is due to the uniform distention of the splenic spaces by red blood cells. One of the most striking features in the section is the numerous collections of parasites which are dotted everywhere throughout the spleen, there being a dozen or more collections to every oil immersion field. In the splenic spaces they are frequently contained within large mononuclear cells, which are either free in the sinus or attached to its wall (Fig. 10); the greater number of parasites are in the latter locations. There are many parasites in groups of two or three dozen free and intracellular throughout the reticulum of the loose adenoid tissue. The splenic spaces are relatively free from lymphoid cells. The cells which are present are large mononuclear in type with round or horseshoe nuclei and there are a few polymorphonuclear leucocytes. The Malphigian corpuscles are extensively invaded by parasites which appear to be either free in small groups or enclosed within large mononuclear cells.

Section of Lung Including a Hyaline Nodule.—The hyaline nodule resembles that described in Case I, but the reticulum seems to be a little more fibrous in character and contains relatively fewer cells. The central portions of the nodule contain relatively few parasites but nearer the periphery there are many large endothelial-like cells which contain two or three hundred parasites.

Many of the alveoli are distended, empty and emphysematous, while others are filled with serum or red blood cells. Just outside the margin of the hyaline nodule the alveoli are emphysematous and filled with red blood cells and frequently with three or four or
more large cells with mononuclear eccentric nuclei and achromatic chambers filled with parasites. These infected cells are from 18 to 30 μ in diameter with round or slightly oval nuclei 5 μ in diameter.

**Bronchiole.—** This section of one of the larger bronchioles is normal and no parasites could be detected anywhere.

**Peribronchial Lymphnode.—** This node showed several large areas replaced by dense fibrous tissue in the periphery of which there was a good deal of black pigment. Here and there where the lymphoid tissue still remained there were large cells sometimes appearing to form part of the reticulum and there were also endothelial cells filled with parasites bulging out into the lymph sinuses.

**Ulcer of Ileum.**—The peritoneum and muscle wall are normal but the mucous membrane is pushed away from the muscle wall by an oval mass, 1 by 2.8 mm. in area, of large round and oval cells, most of which have a large amount of eosin-staining cytoplasm, more or less replaced by parasites. The periphery of this nodule nearest the lumen of the intestine is denuded of mucous membrane almost to the depth of the muscularis mucosa. Under the high power there is seen to be an extraordinary invasion of certain new cells in the nodule by parasites. The invaded cells closely resemble the invaded endothelial cells of the liver. They are 15 to 30 μ in diameter and their round or oval nucleus, which is generally pushed to one side, is 4 to 6 μ in diameter. Right beside these cells are other smaller cells from 6 to 10 μ in diameter with eccentric small round, oval, or mitotic nuclei, which are apparently proliferating in the lymph spaces of the nodule and which have more deeply staining cytoplasm. The nucleus of the invaded cell is either eccentric or has been pushed to one side and the enclosed parasites are clustered in an achromatic chamber similar to infected cells in other viscera. This achromatic chamber has no definite membrane and is surrounded by the eosin-staining cytoplasm of the cell. Some of the invaded cells did not show this achromatic chamber, but merely a cluster of parasites imbedded in the cytoplasm of the cell (Fig. 11). In this section the blood vessels were not involved, but the lymph spaces everywhere in the nodule showed an invasion of their lining endothelium by parasites. While most of the microorganisms were intracellular, quite a large number was apparently
Morphology of Histoplasma Capsulatum.

free. The epithelium of the glands of Lieberkühn was everywhere free from invasion by parasites, yet the inter-glandular connective tissue stroma up to the basement membrane was crowded with them (Fig. 6). The denuded surface of the ulcer was rich in infected cells and there were detached cells lying free on the surface. The morphology of these free parasites on the surface was the same as those imbedded in tissue—no flagellated forms could be detected. This section shows a more intense invasion by the parasite than that of any other tissue and the parasites apparently had spread along the lymph spaces.

Kidney.—There was some edema. The epithelium of the convoluted tubules was swollen and in places desquamated and cloudy. Most of the nuclei failed to take the stain well. Many of the tubules contained granular debris, desquamated epithelium and occasionally a hyaline cast. There was a slight increase in the intertubular connective tissue and in Bowman's capsule. The space between the glomerulus and Bowman's capsule frequently contained a large amount of coagulated albumin. No parasites could be detected.

CONCLUSIONS.

Histoplasmosis is a fatal infectious disease of tropical America resembling kala-azar of India. It is characterized clinically by splenomegaly, emaciation, irregular pyrexia, leucopenia and anemia. The pathological features are the invasion of endothelial cells in the smaller lymph and blood vessels and capillaries by enormous numbers of a small encapsulated microorganism (Histoplasma capsulatum) causing necroses of the liver with cirrhosis, splenomegaly, pseudo-granulomata of the lungs, small and large intestines, with ulceration of the latter, and necrosis of lymph nodes draining the injected viscera.

The disease is caused by a small round or oval microorganism 1 to 4 μ in diameter possessing a polymorphous, chromatin nucleus, basophilic cytoplasm and achromatic spaces all enclosed within an achromatic refractile capsule.

The microorganism differs from the Leishman-Donovan body of kala-azar in the form and arrangement of its chromatin nucleus, and in not possessing a chromatin rod.
The distribution of the parasite in the body is by the invasion of contiguous endothelial cells of the smaller blood and lymph vessels and capillaries, and the infection of distant regions by the dislodgement of infected endothelial cells and their transportation thither by the blood or lymph stream. Thus the skin, intestinal and pulmonary nodules may be due to secondary distribution of the parasite.

The microorganism apparently lives for a considerable period of time in the tissues, because in the older areas of necrosis there are myriads of parasites all staining like recent organisms.

The mode of infection and the portal of entry are unknown; these together with the zoological status of the microorganism may yet be ascertained by physicians living in less salubrious regions of tropical America than Panama and in those not yet disturbed by the sanitary.

EXPLANATION OF PLATES XIX-XXIII.

Fig. 1. Free parasites in film preparation from lung of Case I. Magnified 2,000 diameters. Beside the parasites is a cell containing granules.

Fig. 2. Same magnified 3,000 diameters.

Fig. 3. Mononuclear cells from lungs of Case I, containing many parasites. Magnified 1,000 diameters.

Fig. 4. Pulmonary nodule from Case I. Low power.

Fig. 5. Peripheral sinus in lymph node containing phagocytic mononuclear cell enclosing many parasites. Case I. Magnified 1,000 diameters.

Fig. 6. Section of ileum showing parasites in connective tissue surrounding crypts. Case I. Magnified 1,000 diameters.

Fig. 7. Necrosis of the liver. Case III. Endothelial cells containing large numbers of parasites. Magnified 1,000 diameters.

Fig. 8. Periportal cirrhosis. Case III. Many parasites in large cells within new connective tissue. Magnified 500 diameters.

Fig. 9. Film preparation from the liver. Case III. Endothelial cells containing many parasites. Magnified 1,000 diameters.

Fig. 10. Mononuclear cell in blood space of spleen. Case III. Magnified 1,000 diameters.

Fig. 11. Nodule in intestine. Case III. Endothelial cells containing many parasites. Magnified 1,000 diameters.

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