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A Protozoön General Infection Producing Pseudotubercles in the Lungs and Focal Necroses in the Liver, Spleen and Lymphnodes

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In this paper, Samuel T. A. Darling (1872–1925), at the time (1906) a pathologist in the Panama Canal Zone, described for the first time a case of histoplasmosis on autopsy in a patient thought to have died from tuberculosis. He considered the agent, whose microscopic morphology he described very thoroughly, to be a parasite with a predilection for endothelial and epithelial cells, apparently because it showed what he interpreted as flagella. This “parasite,” however, appeared morphologically quite different from “…those found by Leishman, Donovan, Marchand, Ledingham, and Wright.” The scattered focal necroses in liver, spleen, and lymph nodes also differed microscopically from tuberculous lesions. It remained for H. da Rocha-Lima (Arch. Schiff-Tropenhyg. 16:79–85, 1912) to conclude, based on comparative tinctorial and histological studies, that Darling’s “protozoön” was a fungus. The disease was soon found to occur in Minnesota, but all diagnoses were made post mortem until Dodd and Tompkins reported the first intra vitam diagnosis in 1934 (see C. W. Emmons et al., Medical Microbiology, 3rd ed., Lea & Febiger, Philadelphia, Pa., 1977). In the same year, W. A. De Monbreun (Am. J. Trop. Med. Hyg. 14:93–125, 1934) was able to culture the organism in both yeast and mycelial phases. Its soil saprophytism was detected much later (C. W. Emmons, Public Health Rep. 64:892–896, 1949) and the disease in bats and the role of avian habitats were ascertained a few years later still (C. W. Emmons, Public Health Rep. 73:590–595, 1958; L. W. Ajello, Public Health Rep. 79:266–270, 1964). Histoplasmosis is still a disease of considerable importance in the Western Hemisphere and has recently also been observed as an opportunistic infection in patients with AIDS.

ALEXANDER VON GRAEVENITZ

A PROTOZOON GENERAL INFECTION PRODUCING PSEUDOTUBERCLES IN THE LUNGS AND FOCAL NECROSIS IN THE LIVER, SPLEEN AND LYMPH NODES.

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On Dec. 7, 1905, while examining smears from the lungs, spleen and bone marrow in a case that appeared to be miliary tuberculosis of the lungs, I found enormous numbers of small bodies generally oval or round. Most of them were intracellular in alveolar epithelial cells, while others appeared to be free in the plasma of the spleen and rib marrow. Tubercle bacilli were absent. The following is an account of the case:

Patient.—C. D., negro from Martinique, aged 27, occupation carpenter. Here and there, Paracaima, a village in the Canal Zone. History.—The patient had been a resident of the zone three months. While in Martinique he had suffered from some mental disturbance. His present illness dates from Sept. 15, 1905, when he complained of fever and vomiting.

Condition on Admission to Hospital.—On entering Ancon Hospital, Dec. 5, 1905, he was mildly delirious and incoherent. Lungs were clear; abdomen was scaphoid; spleen was enlarged.

Hemoglobin: 60 per cent. (Daré's).

Feces: Negative.

Temperature: On admission, Dec. 5, 12:30 p.m., 101; pulse 120; Dec. 6, 8 a.m., 95; pulse 90; 4 p.m., 98, pulse 100.

The patient died Dec. 6 at 11:30 p.m.

AUTOPSY.

December 7, 8:30 a.m.

Macroscopic and Microscopic Examination.—Body of negro, moderately emaciated; length, 5 feet 8 1/4 inches; inter nipple distance, 7 3/16 inches; rigor mortis was plus.

The color on opening thorax was suggestive of pulmonary tuberculosis. The right and left pleura were free. There were numerous red blotches (echymoses) beneath the visceral pleura of both lungs 8 mm. in diameter. Many small nodules could be felt under the visceral pleura.

The lungs on section were found studded with pale gray parenchymal miliary tubercles from 2 to 5 mm. in diameter. The lungs were heavier and more voluminous than normal. The tubercles were not as closely packed or so numerous as is often found in miliary tuberculosis, and the general color of the lungs was bright red.

The peribronchial lymphnodes contained a few small soft, recently caseated tubercles. The nodes were enlarged and pigmented.

Heart: This organ was small and normal.

Liver: The liver was enlarged and pale, and there was slight atrophic cirrhosis.

Spleen: This was enlarged to three times the normal in size; the pulp was very firm. The malpighian bodies were distinct. Here and there were a number of small yellow nodules resembling tubercles.

Kidneys: There were a few depressions in a cortex diminished to 8 mm. in depth.

Pancreas: Normal.

Bladder: Normal.

Rib bone marrow: Normal and dry.

Brain: The pineal gland was slightly edematous and more generally adherent to the cortex than normal. The calvarium was very thick.

Intestines: Several specimens of Tricophyton dispar were found in the cecum. There were a few small superficial cubicular ulcers from 2 to 4 mm. in diameter in the cecum and ileum.

The mesenteric lymph nodes and those at the hilum of spleen were enlarged and pale.

Bacteriologic Examination.—Spleen smears were negative for malarial parasites or pigment. Oral and round bodies were free in the plasma.

In rib bone marrow smears there were traces of intracellular malarial pigment. A number of bodies similar to those in the spleen were seen.

In lung smears tubercle bacilli were absent.

There were myriads of intracellular and extracellular bodies similar to those found in the spleen and the marrow.

A moist cellophane preparation from intestinal ulcers showed motile amebae.


APPEARANCE OF THE PARASITE IN SMEARS.

Lungs: This specimen was stained by carbol-fuchsin and Gabbett's methyl blue, overstained with polychrome methyl blue, and washed with eosin.

The polychrome blue was prepared as follows: Methyl blue, pure, medc. Grith...: g. 1. Sodium carbonate, pure..........................g. 5 Distilled water.........................................g. 100.

This was placed in thermos for one week and kept at room temperature for six months.

The excess of blue was removed by washing the smear alternately with alcoholic solution of eosin (0.5 per cent in 20 per cent. ethyl alcohol) one second and distilled water a few seconds, until the internal structure of the parasites showed plainly.

The parasite is oviform or round, and is surrounded by a clear refractile non-staining rim, in thickness about 1/5 the diameter of the parasite. This refractile rim is present in all smears, whether previously treated with acid blue or not. The structure is not homogeneous, but consists of a faintly staining substance and a deeply staining core; a clear space or spaces; and chromatin granules. The chromatin granules are generally single, sometimes two or more are counted. One large parasite appeared to have six such dots of chromatin. The granules are often situated in a clear non-staining zone at one side of the darker staining substance; at other times they are situated on the margin or within this substance; and also frequently appearing in the clear refractile capsule. The chromatin granules are generally dot shaped, very rarely elongated. Occasionally two chromatin dots placed together formed a red form.

The clear space or spaces resemble vacuoles; at times they resemble the clear non-staining spaces seen in fibrin embryos and typhusomes. The staining substance almost entirely fills the capsule or refractile rim of the parasite. The circular contour of the staining substance is at times broken on one side or place by the clear non-staining zone.

This zone varies in shape, size, and in its relation to the staining substance; being circular, oval, or irregular in form: being three-fourths the size of the entire parasite, or at times barely perceptible on account of its minuteness; being centrally located or eccentric; and being single or multiple—two or three.

In size the parasites are from 1 to 4 microns through their greatest diameter; commonly this diameter is 3 microns.

The parasite appears to divide by fission into two equal or unequal elements. One parasite appeared to be dividing into two equal elements. Several parasites with chromatin dots scattered through their substance appeared as pre-segmenting bodies—ready to divide into five or six elements. Occasionally a smaller parasite may be seen close beside a larger one, as though separating from it, the smaller one being about 1 micron in diameter.

Although oval or round in outline, the staining substance,
together with the clear non-staining zone and chromatin granules, give a varying picture, depending on the point of view. Forms suggesting the appearance of familiar objects, such as the eye, a shield, a conch shell, a bullet, or a shuttle are seen. The resemblance of certain parasites to a mammalian embryo in "fetal attitude" is very striking.

In the lung smears the parasite is apparently always intracellular, and the cells contain from 10 to 100 or more parasites. The appearance of free parasites is probably due to the squeezing and breaking up of infected epithelial cells by pressure in making the smear. One unbroken alveolar epithelial cell occupied one-third the diameter of the field, 1/11 oil im. No. 1 oc. B. & L. Parasites had invaded the cell nucleus as well as the cytoplasm, and it was estimated that this cell contained more than 300.

Spleen and rib marrow smears showed fewer parasites, two or three to a field, and they appeared to be extracellular. The seen within the capillaries. The epithelial cells of the alveolar walls are desquamating or completely shed. In places there appears to be merely a single layer of endothelial cells separating the circulating blood from the alveolar contents. The alveoli are seen to be filled with red blood corpuscles and generally having a washed out appearance; red blood corpuscles and serum; or red blood corpuscles, serum, and large swollen alveolar epithelial cells containing many parasites. Polymorphonuclear leucocytes are rarely observed in the alveolar contents; a few mononuclear elements are noted. There are no tubercles. The pseudotubercular areas are made up of alveoli with broken, distorted, or collapsed walls, containing many alveolar epithelial cells distended by parasites. Small vessels or capillaries are seen to pass through the pseudotubercles, but there are no evidences of the hemorrhages seen in other alveoli. Within these areas there are numerous numbers of parasites generally contained within epithelial cells—rarely free. The nuclei of invaded cells stain well, though often more faintly than the normal. The cytoplasm of badly infected cells is wanting, and there are numerous distended epithelial cells devoid of cytoplasm and parasites. The infected cells have a distinctly staining rim of cytoplasm, even when their nucleus and cytoplasm are gone.

Liver: There are numerous faintly staining areas ranging in size from that of a single glandular epithelial cell to those one-third the size of a lobule; in which the liver cells and endothelial cells of the portal capillaries are completely transformed by invading parasites. In the larger areas the cytoplasm and nuclei of the invaded cells have disappeared or do not stain. There is a mass of debris, imbedded in which are myriads of parasites. In places the liver cells are normal, in others they have suffered cloudy change. In these latter localities there appears to be a stasis of blood in the portal capillaries due to occlusion of capillaries by enormously distended endothelial cells filled with parasites. The red blood corpuscles are here "washed out."

There is a distinct primary invasion of liver cells in places, although oftener it would seem that many liver cells become invaded after they have had their nutrition cut off by infected overlying endothelial cells.

Around the portal spaces the connective tissue is increased in amount and there is round cell infiltration. The bile capillaries and their epithelium are normal.

Spleen: The splenic spaces are greatly engorged with red blood corpuscles. The connective tissue is moderately increased, its cells are swollen, cloudy, and at times contain parasites. There is cloudy swelling of cells in small areas here and there, and many of these cells contain parasites. There are also numerous free parasites.

Lymph node from hilum of spleen: The cortical follicles and medullary cords of the dense lymphoid tissue are, with the few exceptions noted, below normal. The capsule and reticulum throughout the node are the seat of degenerative changes. The reticulum of the loose lymphoid tissue envelopes many large mononuclear cells possessed of distinctly staining nuclei, and containing many parasites.

There are two cortical follicles, and portions of a medullary cord which have undergone cloudy swelling and necrosis, and the debris of which are cells containing parasites. The margins of these areas show beginning degenerative changes; many fragmented nuclei are seen, as well as mononuclear cells distended by parasites.

Peribronchial Lymph node: This node contains several old fibrous-caseous tubercles, and one giant cell. The reticulum and capsule of the node are greatly thickened in places. A lymph vessel beneath the capsule contains mononuclear cells infected by parasites.

There is seen to be a general infection by a parasite having a predilection for endothelial and epithelial cells. The lesions are those of scattered focal necroses of liver, spleen and lymphnodes, with foci of catarrhal pneumonia and hemorrhages in the lungs, in which the lungs play a very passive part, there being absolutely leptocytic infiltration of the military pneumatic nodules.

The infection was a fatal one, there being no other
lesions sufficiently grave to have caused death. The anatomical diagnosis of tuberculosis not being confirmed on examination of sections, save in peribronchial lymph-nodes.

The parasite, as studied from smears, presents certain resemblances to those found by Leishman, Donovan, Marchand, Ledingham and Wright, but the differences are so marked and the lesions so unusual that I feel the case is a unique one.¹

For the parasite the name *Histoplasma capsulata* is proposed.

I wish to thank Acting Chief Sanitary Officer Dr. H. R. Carter for his kind permission to publish this report.

¹ Since writing this article I have found the parasite in a second case.