THE PATHOLOGY OF EXPERIMENTAL YELLOW FEVER IN THE *MACACUS RHESUS* *

II. MICROSCOPIC PATHOLOGY

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INTRODUCTION

This report on the histopathology of experimental yellow fever is based on the microscopic study of tissues of thirty *Macacus rhesus* monkeys fatally inoculated with the Asibi strain of the virus.[1] All these animals are included in the report on the gross pathology[2] found in the sixty-eight monkeys of the same species.

The virus was transmitted in various ways to the animals microscopically studied. These methods may be grouped in the following manner:

Blood from patient (Asibi)	1
Blood and organ emulsion from monkey	1
Blood from monkey	8
Blood from monkey applied to scarified skin	1
Monkey serum filtrate (Berkefeld N)	1
Monkey serum filtrate (Seitz asbestos)	1
Mosquito (*Aedes aegypti*) transmission	16
Emulsion of mosquitoes (*A. aegypti*)	1

Monkeys were examined and tissues preserved as soon after death as possible, because of rapid postmortem changes. The necropsy was done at once in the case of twenty animals, within one hour after the death of seven, and a few hours postmortem in three. In a few instances, chloroform was used to hasten the end of moribund animals in order to make the postmortem examination by daylight and, compared to the large number of animals not so treated, the small amount of chloroform needed did not seem to alter the microscopic pathology.

The diet of all monkeys has been rice, oranges, bananas, bread, evaporated milk and water. For purposes of comparison, the tissues of two apparently normal and several rhesus monkeys dying from natural causes (dysentery, tuberculosis and undetermined) were available.

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The tissue fixatives employed were 10 per cent formalin and Zenker's fluid and the staining method was routinely hematoxylin and eosin; occasionally van Gieson and Giemsa preparations were studied. For the demonstration of fat, frozen sections were stained with scarlet red and counterstained with hematoxylin.

Pathologic Histology

Liver: This organ furnishes the most extensive and constant pathology and shows chiefly fatty degeneration and necrosis.

No fat is found in one normal rhesus and in the other, it occurs as large droplets sparsely sprinkled through the lobule. In monkeys dying from natural causes, there is only a small amount of periportal fat. In paraffin sections of the experimental tissues, large and small vacuoles are evident, especially in the normal or moderately altered liver cells, such as in the periportal region. In frozen sections, fat is demonstrated in every liver. In the majority of specimens, it is present in extreme amounts, occurring as minute to large droplets in practically every parenchymatous cell. In numerous instances, more fat, usually as larger droplets, is found in the periportal and central zones of the lobule. In one liver (No. 304) in which the necrosis is mild, fatty degeneration is limited to the midzone.

In the normal liver, the parenchymatous cells are regular, polyhedral and arranged roughly in columns; the neutrophilic cytoplasm is not uniformly stained and gives a positive test for glycogen (Best's carmine stain). The cytoplasm of the liver of monkeys dying from natural causes is smoothly stained and neutrophilic. In contrast to these control animals, the hepatic cells of experimental monkeys are not arranged in columns in the affected regions, but are jumbled, rounded and irregular in size and shape. No glycogen is found in these sections (one monkey tested).

Early degenerative changes are represented by the loss of the normal cell relation and the presence of mild to marked eosinophilic-staining properties. More advanced stages of necrosis appear as acidophilic, coarsely granular cytoplasm in distorted cells. Finally, disintegration takes place and the cytoplasm is either intensely or poorly stained, and granular; the cell wall may be entirely indefinable. In about two-thirds of the livers, hyaline degeneration is one of the forms of cytoplasmic change. Hyalin is seen as small, irregular

homogeneous, well-stained masses within the cell and occurs in only a relatively few cells of the section.

Nuclear changes coincide with the changes of the cytoplasm. Large, poorly stained, vesicular nuclei with prominent nucleoli are found in the necrobiotic liver cells. In such nuclei, minute, dull red granules are often found. They were present in half the thirty specimens studied; in an additional six specimens, this type of nuclear change is less often present. Occurring in half the livers of this group, variable numbers of nuclei appear as small, round, intensely acidophilic dots. This form of degeneration is usually seen in the comparatively rare, small liver cell having smooth, intensely stained cytoplasm. The nucleus finally disappears in advanced necrotic cells either by lysis or karyorrhexis. In eleven specimens, karyorrhexis is common or marked, while in five others it occurs relatively infrequently. When karyorrhexis is marked, scattered nuclear débris is conspicuous in the hepatic cells throughout the section. Mitosis is not found.

The degree of necrosis varies only slightly among the specimens and in only one instance (No. 304) is it limited to the early stages of degeneration. No effort has been made to determine the variation in degree of necrosis throughout the liver, but there is reason to believe that such exists. The variation in any one section, however, is negligible, all lobules being equally affected.

The extent of necrosis and necrobiosis in the lobule, on the other hand, is not constant among the specimens except that the midzone is always attacked. In two cases, the midzone alone is necrotic, mild in one (No. 304) and extreme in the other (No. 312). In one-third of the livers a wide intermediate zone encroaching upon the periportal and central zones is involved; in an additional third, parenchymatous cells of all zones are degenerated, leaving only a fringe of intact but vacuolated cells about the portal region and central vein; and in the other instances, a border of periportal cells alone is undegenerated. In the majority of cases of extensive necrosis, the most extreme changes are midzonal. Under such circumstances, the most numerous necrotic cells are in the intermediate zone, while toward the limiting structures the necrotic cells, mingled with the necrobiotic cells, decrease and the latter type increase in numbers. The same is true of necrobiotic cells in relation to the intact and normal cells. In no case is either central or periportal necrosis alone encountered.

In spite of disintegration of parenchymatous cells, no collapse of cell space is evident and lobules retain their normal shape and size.

Sinusoids are easily followed except when distorted in extremely necrotic areas. Their lining cells are rarely enlarged and only occasionally phagocytic. The nuclei of these cells are well stained and sometimes comprise the only intact nuclei of a necrotic region. Congestion is not a conspicuous feature and when present is irregular and mild. No thromboses are evident. Hemorrhage is rare and is found to an appreciable degree in one instance in the midzone and in another about the central vein. On the other hand, a few to numerous scattered red blood cells are seen to be extravascular in one-third of the specimens.

Portal areas are commonly not remarkable aside from mild and variable lymphocytic infiltration. Often endothelial leucocytes and occasionally polymorphonuclears are found in these regions and in one instance necrosis is obvious. There is no fibrosis.

In this organ, inflammatory cells are generally present in the necrotic regions but their presence and numbers do not depend on the degree or extent of parenchymatous necrosis. In twenty-four specimens, scattered polymorphonuclear leucocytes are in the sinusoids, and in three of these, they occur in foci as well; in half of these twenty-four instances, these cells are also among or in necrotic liver cells, but not in large numbers. In about the same number of animals, not always, however, in the same specimens, endothelial leucocytes are found, both intravascular and extravascular. In the latter situation, they are occasionally large and phagocytic, sometimes containing acidophilic granular débris. While inflammatory cells commonly accompany the necrosis of parenchymatous cells, their occasional complete absence indicates that they do not constitute an essential part of the pathology. Lymphocytes and eosinophiles are not found.

Kidney: Fatty degeneration is not demonstrable in this organ of the normal and uninoculated monkeys. In paraffin sections of numerous experimental tissues, fine vacuoles are seen near the base of epithelial cells of cortical tubules. In frozen sections stained for fat, large amounts of this substance are demonstrated as minute to small droplets in the tubular epithelium of twenty-seven of the thirty specimens. The convoluted tubules show the most advanced fatty degeneration and when less than extreme amounts of fat are present,

the straight tubules are spared. Fat is not stained in the glomerular structures or in parts other than the tubular epithelium.

In non-experimental animals, the renal tubular margin is slightly irregular, but the lumen is distinctly patent. In experimental sections, the epithelial cells of convoluted tubules, more than of straight tubules, are swollen, slightly granular and occasionally acidophilic. In twenty-three specimens, this acute degenerative change is marked almost to the occlusion of the lumen of the convoluted tubules; it is mild in three others and inconspicuous in three. Necrosis is not extensive in these monkeys; however, a relatively few to numerous necrotic epithelial cells of cortical convoluted tubules are evident in the majority of cases. Such cells are granular, disintegrated and without nuclei, and in the presence of one or two necrotic cells in the cross-section of a tubule, the margin is ragged.

The nuclei of these cells showing cloudy swelling are usually well preserved, although in a few instances some are poorly stained. Ten specimens demonstrate pyknotic nuclei of epithelial cells, which are more generally found in straight than in convoluted tubules. Karyorrhexis is relatively uncommon and is conspicuous in only two instances.

No inflammatory cells are found in kidney sections, either in degenerating cells or in the tubules. Congestion is not marked and when present, occurs mildly and irregularly. There is no indication of hemorrhage and no red blood cells lie in the tubules.

Tubules of the cortex and medulla as a rule contain varying amounts of granular or circular débris. Few to many granular and hyaline casts are in straight and collecting tubules of about one-half the specimens. The two varieties are not always in the same animal, although as a rule they are coincident. Sometimes they are distinctly bile-stained.

In six animals, deposits of hematoxylin-stained material are seen in collecting tubules and less frequently in convoluted tubules. They are best demonstrated in formalin-fixed tissues as either clumps of minute granules, larger solid masses, or discs. Their color varies from slate-blue to almost black. In frozen sections counterstained with hematoxylin, they are conspicuously darkly stained. After treating paraffin sections with silver nitrate according to Klotz' method, these deposits appear dark brown or black. Immersion in a weak solution of nitric acid before staining makes it difficult to

demonstrate the deposits with hematoxylin; those found are only faintly colored, whereas cell nuclei are not altered in their staining properties. With these findings and the demonstration of form, location and hematoxylin-staining, it seems that such casts are calcareous deposits. At least one source of this variety of cast may be a form of degeneration of the epithelium of collecting tubules, since one can trace similar intracellular masses through the changes of the cell to its necrosis and disintegration. Sometimes these calcareous deposits form a rough ring about the lumen of the collecting tubules, either within or on the surface of necrotic cells or in the entire absence of epithelium.

Glomerular capillaries are sometimes congested and their spaces occasionally contain a small amount of granular débris; otherwise these structures are not remarkable. The renal capsule and pelvic mucosa are unaltered.

Heart: In comparison to the normal controls, paraffin sections show little change in cellular structure. In a few specimens, the fibers are irregularly stained, the cross-striations are indistinct and the longitudinal fibrils are prominent. Inflammatory cells are not present in any part. Congestion of small vessels is noteworthy in six cases and mild in two others. Diffuse recent hemorrhage without reacting inflammatory cells is found under the endocardium and among the neighboring muscle fibers in one animal.

In frozen sections stained for fat, it is demonstrated as very minute droplets, usually distributed unequally among the fibers. In six specimens the amount is extreme, nearly all the fibers being involved; in sixteen the fatty degeneration is marked; in five it is mild; no fat is found in two; while in one case the tissue is not available for study.

Spleen: Sections of normal rhesus spleens show much lymphoid tissue with active germinal centers, a relatively small amount of pulp and almost collapsed blood spaces. In the experimental animal, the lymph nodules are regularly much reduced in size and widely separated by marked to extreme congestion. In three specimens, there are distinct zones of hemorrhage within numerous nodules.

The germinal centers are small, devoid of lymphoblasts and are composed of large, elongate stroma and endothelial cells. These remains of the germinal center, present in about half the thirty specimens, contain small numbers of lymphoblasts in seven instances.

A conspicuous finding in all but four specimens is necrosis in and about the germinal centers, which is manifested by disorganization, swollen disintegrating cells and much nuclear débris. Even in the absence of the germinal center, necrotic lymphoid cells of the nodule are often found. Constantly accompanying the necrosis, there is a response on the part of the endothelial cells, without, however, any polymorphonuclear infiltration. The endothelial cells probably have a local source and assume phagocytic activities, since one can trace such cells, marked by contained brown pigment granules, from their location in the germinal centers of the normal spleen through changes responding to the necrosis of the lymphoid cells, to their own degeneration. Normally they are flat or rounded; in experimental specimens they are often swollen, vacuolated and phagocytic; and in about one-fourth of the specimens, they show necrosis and disintegration.

Satisfactory frozen sections of this organ, stained for fat and counterstained with hematoxylin, were obtained from twenty-eight specimens. After careful search of several sections of the normal spleen, small numbers of fat granules were seen in only two or three endothelial cells of the germinal centers. In the experimental tissues, extracellular granular fat is demonstrated in nine instances in the necrotic areas of the lymph nodules. In the same areas, many endothelial cells containing fine fat granules are found in nineteen specimens and fewer such cells in an additional six tissues.

Not only is there an endothelial response within the lymph nodule, but also about the nodule and in the pulp. In half the tissues of this group of animals, there is a distinct, and in some cases marked, hypertrophy and apparent hyperplasia of the endothelial cells. These cells are large and rounded, their nuclei are commonly weakly stained and occasionally they show degenerative changes. Endothelial leucocytes (free mononuclear cells) are likewise mildly to markedly increased in numbers in about two-thirds of the specimens; they often demonstrate mitoses and rarely are phagocytic. In numerous instances these two classes of cells are especially prominent and numerous in the neighborhood of the lymph nodule.

In the frozen sections no fat is demonstrated in the endothelial cells of the sinusoids of the normal spleen, but in the experimental animals granular fat is stained in many of these cells in about one-third of the specimens.

Polymorphonuclear leucocytes are variable in occurrence. They are conspicuous in the pulp in eleven instances and are in large numbers in six additional cases. As a rule, these cells are scattered and only in one instance are also in foci.

Lymph Nodes: Sections of these structures are available for examination from twenty-two animals. In nineteen cases, they are from the axillary or inguinal regions, and in other instances from miscellaneous sources. While the lymphocytes are not appreciably reduced in numbers, other changes are similar to those in the spleen: the germinal centers are small and often devoid of lymphoblasts; necrosis in the region of the germinal centers is present in over half the specimens and accompanied by enlarged, phagocytic endothelial cells. Several sections of lymph nodules from miscellaneous sources (gastric and duodenal submucous, peribronchial, mesenteric, in the region of the pancreas) likewise show the same degenerative changes.

Phagocytic endothelial leucocytes are common in the sinuses, but are also found in the normal lymph nodes. Congestion is noteworthy in a few specimens, while hemorrhage is not encountered.

Lungs: Recent hemorrhage into small groups of alveolar spaces or in the subpleural region is present in thirteen specimens. This effusion of blood is not massive, no break in the alveolar wall is obvious and an inflammatory reaction does not result. In a few instances when cysts of *Pneumonyssus griffithi* [2] are in the sections, small areas of recent hemorrhage are commonly adjacent. Congestion of alveolar capillaries is not conspicuous, but is found in eight of the thirty specimens and not always coincident with the hemorrhages.

Stomach: Of the twenty-four sections of stomach available for study, only three show small areas of recent hemorrhage in the mucosa and without inflammatory reaction. In an additional three specimens, appreciable numbers of extravasated red blood cells occur in the same location. They are also found on the mucosal surface, usually not coincident with the extravasations in the mucosa, in seven instances. No lesion or erosion is evident in any case, and congestion of mucous or submucous capillaries is neither common nor extreme.

Intestines: Sections of intestine were not examined routinely and only one of the few studied shows an extensive submucosal and less subserosal hemorrhage without inflammatory reaction. No lesion in the mucosa is found.

Adrenal Glands: The outstanding pathology of these structures is necrosis of the cells of the zona fasciculata. This occurs in about half the thirty specimens of the series. The necrosis involving numerous scattered cells to small and large groups of cells, is manifested by loss of staining property, disorganization, and fusion of necrotic cells. Karyorrhexis is common and much nuclear débris is often present. Polymorphonuclears invariably react to the necrosis and are found in large numbers both in the neighboring blood spaces and within the necrotic cells or areas.

Eight specimens show congestion of the small blood vessels. In three cases there are either mild or marked recent hemorrhages in the zona fasciculata and between this layer and the medulla. Almost every specimen, as well as those from the control monkeys, has from two to six peculiar calcareous concretions in a section in the medulla close to the cortex.

Pancreas: This organ is not essentially altered aside from frequent congestion and the acute pancreatitis in one animal (No. 229).

Voluntary Muscle: The variation in staining reaction of the muscle fibers and occasional loss of cross-striations are similar to the findings in the normal rhesus tissue. Likewise, the frequent cysts of Sarcosporidia are present both in the control and experimental animals.

Brain: Six specimens of this organ demonstrate no essential deviation from normal, aside from occasional mild congestion.

Discussion

The type or degree of pathology shown by these animals does not seem to depend on the method by which the virus was transmitted.

Fatty degeneration is a prominent feature in the liver, kidney, heart and spleen. In the first three of these organs, the parenchymatous cells are thus regularly affected, while in the spleen the necrotic areas in the nodules and endothelial cells of the pulp and nodules show a fatty change.

Necrosis is likewise common, involving the liver, kidney, lymph nodules of the spleen and regional nodes, and adrenal glands. Cloudy swelling is more extensive than necrosis, however, in the renal epithelium. Apparently in response to the degenerative changes, polymorphonuclear and endothelial leucocytes are usually increased in the liver, the latter type of cell responds in the lymphoid system,

polymorphonuclears react in the suprarenals and no inflammatory cells are found in the kidney or heart. It seems that these reacting cells are called out by more advanced degeneration, rather than by early degeneration or the presence of the virus, since we find them only in those structures showing marked necrosis, and neither in the kidney and heart nor scattered generally where it might be supposed the virus has been.

Hemorrhage as it occurs in the gastric mucosa, lungs and liver, is recent, without inflammatory reaction and not extensive. In view of the fact that blood effusion is mild and is often found microscopically when not seen in gross, it seems that the extravasation of blood is rather by seepage than by rhexis of a vessel. In two instances (sections of intestine and adrenal gland), however, the hemorrhages are extensive.

No definite evidence is furnished by this study as to the source of the jaundice in these animals. The bile capillaries do not contain inspissated bile and the bile ducts are not remarkably altered. There is a severe disorganization of the parenchymatous hepatic structure, but the endothelial cells of the sinusoids are relatively unaffected. It is noteworthy that although some yellowish brown pigment granules are found in the endothelial cells of the normal splenic pulp, much more pigment is regularly demonstrated in these cells in the experimental monkeys. In frozen sections, the blood spaces are often beautifully outlined by pigment granules.

In the liver parenchyma, an acidophilic, granular type of degeneration is constant and hyaline changes often take place. Nuclei of degenerating liver cells commonly undergo acidophilic changes. While strict midzonal necrosis occurs in only one specimen, the common presence of most extreme necrosis in the intermediate zone and the absence of degeneration of either other zone alone, indicate that the type is fundamentally midzonal and probably begins as such. This view is supported by the fact that the one specimen (No. 304) showing early changes, has undergone fatty degeneration only in the midzonal cells.

The acute degenerative changes of the kidney epithelium and the presence of casts in the tubules indicate the source of the abnormal urine findings of albumin and casts. The occasional presence of lime deposits are likewise of interest.

The sequence of events in the splenic nodule appears to be a dimin-

ution in the number of lymphocytes and lymphoblasts, followed by necrosis of the latter cells and neighboring lymphoid cells, sometimes with fatty changes; the whole is accompanied by a local endothelial activity, and in turn, by fatty degeneration and necrosis of the endothelial cells. Many specimens show small lymph nodules and no trace of germinal centers. While this may be due to the age of the animal, it seems rather that the centers have disappeared in the course of the disease, since at necropsy only a few monkeys were obviously old and microscopic evidence of advanced age is seldom encountered. Lymphocytes are not increased in the blood spaces and the supposition is that the lymphocytes disappearing from the lymph nodules are not diffused through the pulp. The presence of polymorphonuclears is probably in response to the degeneration of endothelial cells.

Special staining for microörganisms in the monkey tissues has been undertaken in three ways, aside from the search in the ordinary paraffin sections. These are Levaditi preparations for spirochetes and leptospiras, Giemsa stains of paraffin sections, and Giemsa, Fontana and other methods of examining smears of fresh tissue. Levaditi preparations, controlled by guinea pig tissues showing large numbers of *L. icteroides*, have not shown any such form in monkeys either running the full course of the disease or killed on the first day of fever (killed monkeys are not included in the series discussed in this paper). Giemsa stains of the paraffin sections have demonstrated bacteria, appearing as postmortem invaders, only in those animals necropsied some time after death. In a limited number of monkeys in which the disease ran a fatal course, in one monkey killed on the first day of fever, and in controls, Giemsa, Fontana and other bacterial preparations were made of fresh smears of the spleen, liver and kidney; no bacterial forms have been found that are not also in the control smears.

Summary

The microscopic pathology in thirty *Macacus rhesus* monkeys fatally inoculated with the Asibi strain of yellow fever virus is described.

In the liver, fatty degeneration, necrosis and nuclear changes are prominent. Polymorphonuclear and endothelial leucocytes are commonly present.

The renal epithelium shows fatty degeneration, cloudy swelling and less extensive necrosis. No inflammatory cells are found. Tubules contain hyaline, granular and, in a few instances, calcareous casts.

Fatty degeneration is an almost constant finding in the muscle fibers of the heart.

The pathology of the spleen includes congestion, diminution of lymphocytes and lymphoblasts, necrosis of lymph nodules and a marked endothelial response in the nodules and pulp. Fat is demonstrated in this organ in the necrotic areas and in the endothelial cells of the nodule and pulp.

Regional lymph nodes likewise show necrosis and endothelial activity.

The lungs and stomach furnish evidence of recent hemorrhage without inflammatory reaction, mild in degree and without obvious lesions in the vessel wall.

Necrosis with a polymorphonuclear reaction is common in sections of the adrenal gland. Mild to marked hemorrhage occurs infrequently.

Tissues so far studied and showing slight or insignificant changes are the brain, pancreas and voluntary muscle.

No bacteria, leptospiras or spirochetes have been demonstrated. The lesions of the various organs may be explained on the basis of a severe intoxication and no nidus of the virus is suggested.

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