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

III. COMPARISON WITH THE PATHOLOGY OF YELLOW FEVER IN MAN

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The preceding papers [1] have presented the gross and microscopic pathology in the *Macacus rhesus* fatally infected with the Asibi strain [2] of yellow fever virus. The purpose of this paper is to compare these findings with those in human cases of yellow fever.

The literature regarding the pathology of yellow fever in man largely concerns this disease as it occurs in the Western Hemisphere. There are, however, a few references available on this subject from West Africa, including articles by Aitken, Connal, Gray and Smith,[3] Aitken and Smith,[4] Klotz and Simpson, [5, 6] and notes by Stevenson,[7] Turnbull,[8] and Boyce.[9]

GROSS PATHOLOGY

In order to obtain more data on the gross pathology of this disease in West Africa, we reviewed the records of thirty-three cases confirmed by microscopic study, contained in the files of the West African Yellow Fever Commission. These necropsy records are available through the courtesy of the medical authorities of Nigeria and the Gold Coast, British West Africa. The postmortem examinations were made in most cases by the local medical officer or pathologist and occasionally by members of this Commission. The microscopic studies were completed by the pathologists at the Medical Research Institutes of Accra, Gold Coast, and Lagos, Nigeria. In addition, all cases but two were examined microscopically by the pathologists of this Commission with complete agreement as to the diagnosis. We wish to express our thanks and appreciation for the coöperation and interest manifested by the medical administrative officers and by individuals who conducted the postmortem examinations, submitted their records for our use and sent specimens for our examination.

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Except for four Syrians and four native Africans, the patients were Europeans, at the time resident in West Africa. A brief résumé of the reported findings in these thirty-three cases follows:

Jaundice: regularly of the skin and a little less often of the sclerae; commonly of the aorta and other large vessels near the heart, cardiac valves, and subcutaneous tissue and fat; frequently of the costal cartilages, renal tissue, and heart musculature; and less often of other parts and body fluids.

Hemorrhage: as petechiae, in a third to a half of the cases, in the skin, pleurae, epicardium and endocardium, mucosa of small intestine, and beneath the renal capsule; occasionally in the peritoneum, papillary muscles of the heart, bladder mucosa and mucosa of colon; and rarely in the subconjunctival tissue, retroperitoneum and mucosa of trachea. Altered blood ("black vomitus") in the stomach in twenty-nine instances and mucosal hemorrhages in twenty-four; blood in the small intestines in twelve, with mucosal hemorrhages in fourteen; in the colon, blood in four and hemorrhages in three. Hemorrhages and congestion in the lungs in about two-thirds of the cases. Evidence of bleeding gums frequently recorded.

Liver: regularly "boxwood" in color, described as yellow, yellowish brown, yellow-khaki, or reddish yellow; sometimes enlarged; and on section, often friable, fatty, mottled by lobular markings, but seldom congested and hemorrhagic.

Spleen: usually enlarged, congested, firm and malpighian bodies often prominent; malaria in one case (a native African).

Kidneys: enlarged and congested in over half the cases; often icteric; cortex swollen, cloudy and fatty; and blood in renal pelvis in four instances.

Heart: often pale or pale brown; sometimes soft, and certain parts hemorrhagic as described under the subject of hemorrhages.

Bladder: occasionally empty but as a rule containing bile-stained urine, positive for albumin, casts and bile.

Other Organs: adrenal glands, normal; pancreas, occasionally bile-stained and congested; lymph nodes, not remarkable or slightly enlarged; brain (one specimen), mildly congested. Four patients were women; uterine hemorrhage was found in two cases, of whom one was known to be menstruating at the time.

Cutaneous jaundice and petechiae were obviously difficult to determine in the four cases of African natives. Likewise, any abnormal

coloration of the liver and fat in these individuals is confused by the pigmentation caused by the common diet of palm oil. It should be noted that the necropsy observations were made by several investigators and in some cases were incomplete. Taken as a whole, however, the above résumé with articles of Aitken and others,[3, 4] displays the fact that in the gross findings, yellow fever as it occurs in West Africa is not essentially different from yellow fever of the Western Hemisphere. It is not within the province of this paper to go into details as to the comparison of these findings in the two hemispheres, but simply to furnish a basis for comparison with the findings in the *Macacus rhesus* to which, thus far, yellow fever has been transmitted only in West Africa. The reader is referred to articles describing the pathology of yellow fever in the Western Hemisphere, by Boyce,[9] Marchoux, Salimbeni and Simond,[10] Marchoux and Simond,[11] Rocha-Lima,[12] and more recently by Noguchi,[13] Elliott,[14] and Muller and Blaisdell.[15]

Comparison of Gross Pathology of Human and Monkey Cases

Icterus: A constant finding at necropsies of human victims of this disease, was also manifest in the monkeys, but to a less intense degree. Thus while the skin of monkeys was not, as a rule, jaundiced and the tarsal conjunctivae not always deeply colored, a yellow or greenish yellow color of the laryngeal cartilage, large vessels near the heart and body fluids was regularly observed, and other parts were irregularly icteric. It is to be noted that the abnormal coloration was the same in man and rhesus, that is, a lemon yellow and not an orange or brown color.

Hemorrhages: These were found in both human and monkey cases in the pleurae, lungs, gastro-intestinal tract and gums, but were rarely observed in this series of animals in the endocardium and not seen in the skin, peritoneum, retroperitoneal and perinephritic tissues, epicardium, bladder, liver or kidneys. Petechiae were smaller in the animals than were commonly found in man, but in both the hemorrhages were of recent origin. Altered blood in the stomach deserves special mention because of its prominence. In the series of human cases it occurred in almost every instance, while in the monkeys it was found in one-third of the specimens and

was remarkably similar in appearance. As in man, "black vomit" was often found unassociated with mucosal hemorrhages. Likewise, the intestinal contents were colored reddish brown or black by admixed altered blood both in human cases and in monkeys, with perhaps a greater tendency to mucosal hemorrhages of the duodenum in man.

Lungs: Hemorrhagic changes were present in the lungs of the rhesus as well as of man, and the absence of congestion in the experimental lungs, when the necropsy was done immediately after death, probably indicates a difference in posture.

Liver: The color, pallor and fatty appearance of the liver were strikingly similar. The friability and lobular mottling of the liver tissue, when observed in humans, was the same as in the monkeys. Hemorrhagic areas were absent in the animal livers but there was an agreement in the usual dry, bloodless condition of the sectioned tissue. In the human, the liver was reported to be enlarged in some cases; in experimental yellow fever, we felt that the slight enlargement in some instances was within normal limits.

Spleen: The gross findings in the spleen were alike as regards enlargement, congestion and firmness. There was this difference, however: whereas in the human, the malpighian bodies were often prominent and contrasted with the surrounding congested tissue, in the monkey these follicles were usually very small and poorly outlined. It is of interest that in the records of several human necropsies, the lymph follicles were reported as indefinitely outlined and barely visible.

Kidney: Acute degenerative changes in the kidney as evidenced by an enlarged organ and a cloudy, swollen cortex were found in both classes of specimens. Congestion, often referred to in the human cases, was not a conspicuous feature in the monkey kidneys, and hemorrhage was likewise not seen in the experimental tissues. Icterus, common in the monkeys, was often recorded as being present in human kidneys.

Heart: The heart was sometimes pale and yellowish both in man and in the rhesus and the weight was similarly unchanged. As previously mentioned, the cardiac surfaces were often icteric, but the tendency to hemorrhage in these surfaces was greater in human cases.

Urine: The urinary findings were identical, but hemorrhages were not found in the bladder mucosa of monkeys.

The organs that were spared or showed minor changes were the same in human and animal cases: adrenal glands, lymph nodes, pancreas, organs of the neck, voluntary muscle and brain. Data on the condition of human genitalia are incomplete but in the monkey only jaundice of mucous surfaces was apparent.

Summary

It is evident that similar pathologic processes have taken place in the organs of human and rhesus cases of yellow fever. Jaundice, hemorrhage of various parts, "black vomit," pallor, and fatty necrotic changes in the liver, acute degeneration of renal parenchyma, splenic congestion and urinary findings were present in both man and monkey. Although variation existed as to the degree of intensity or extent of involvement of parts, qualitatively the parallelism was striking as regards the icteric color, the recent hemorrhages, and the appearance of the liver, kidney and spleen.

Microscopic Pathology

The histologic pathology of yellow fever has been a subject of considerable study, with particular attention paid to the striking lesions in the liver. Councilman [16] early described the hyaline bodies found in the liver cell. Rocha-Lima [12] has emphasized the midzonal location of hepatic necrosis, while Marchoux and Simond [11] have shown the tendency to fatty degeneration throughout the organs. Seidelin [17] points out that the liver is the seat of constant pathology, in which there is variation in extent of parenchymatous involvement, but in which disorganization of tissues, necrosis and fatty degeneration are conspicuous. As regards microscopic studies of this disease occurring in West Africa, reference should be made to the articles previously cited, by Aitken and his collaborators,[3, 4] Stevenson,[7] Turnbull,[8] and recently by Klotz and Simpson,[5, 6] who have contributed especially to the study of the spleen in this disease.

We have had the opportunity of studying the tissues from thirty cases of yellow fever occurring in West Africa (Nigeria, Gold Coast, Gambia and Senegal). These specimens did not always include all the organs, but at least the organs showing the changes essential for

diagnosis were available. In addition, tissues from ten yellow fever cases occurring in the Western Hemisphere were studied in Toronto, Canada, through the kindness of Dr. Oskar Klotz. From these studies, we can concur in the opinion of Klotz and Simpson,[5] that "no fundamental difference was to be noted in the pathology of fatal yellow fever cases of West Africa and the Americas." It is not the purpose of this paper to deal with a comparison of the pathology of this disease in the two hemispheres, but to compare the pathology of the monkey tissues with that discussed in the literature and studied by us in human cases. The reader is referred to the works of the various investigators enumerated and to an article in preparation by Klotz ("A fuller study of the comparative pathology — will be taken up in another report " [5]) not yet at hand.

Liver: As in the human, the liver (Figs. 1 to 4) of experimental animals shows the most striking lesions. There is the same type of fatty degeneration with the finer particles of fat often in the midzone; in the animal, however, the quantity of fat stained is more than in most human cases. Likewise, a similar type of necrosis exists in the two sets of tissues, both as to being a granular acidophilic form of degeneration of the parenchymatous cells, and as to demonstrating a midzonal location. In the two sets of tissues, the extent of involvement varies from a strict midzone to inclusion of cells almost to the limiting portal area and central vein, but in no instance has there been seen either a periportal or central necrosis alone. The accompanying hyaline type of degeneration is more conspicuous and common in the human than monkey liver. Both man and rhesus livers show jumbled and irregular, necrotic and completely disintegrated cells, with loss of trabecular arrangement but no loss of cell or lobular space. Nuclear changes are similar, with probably more karyorrhexis in experimental specimens and no acidophilic granules in degenerating nuclei in human cases; there is found, however, the same small compact acidophilic nucleus in some acidophilic cells.

The endothelium of the sinusoids is preserved in the rhesus as well as in man and the Kupffer cells are similarly little changed, although more frequently swollen and phagocytic in man. Numerous specimens of monkey liver show numbers of extravasated red blood cells, and only two of the thirty livers studied demonstrate hemorrhages of appreciable degree. It is much more common to find hemorrhages as well as extravascular red blood cells in human specimens. Inflam-

matory cells, of the polymorphonuclear and mononuclear types, are found more regularly in the experimental tissues both intravascular and extravascular. Occasionally, however, they are entirely absent and, conversely, are sometimes found in human cases.

The portal areas of rhesus more than in human livers show variable numbers of lymphocytes and endothelial leucocytes, but the inconstancy of these cells prevents any significance being attached to them. Bile ducts are regularly normal and no inspissated bile is seen in bile capillaries of either set of tissues.

Spleen: The spleen (Figs. 5 to 8) presents interesting comparative pathology in that the following characteristics are similar: congestion, small lymph nodules, and an endothelial response in the nature of enlarged endothelial cells of the pulp, especially about the nodules, and an increase in free endothelial leucocytes. While in man the germinal centers are generally lacking and rarely show necrosis, in the rhesus remnants of the centers are commonly present and necrosis with large phagocytic endothelial cells is regularly seen. Polymorphonuclear leucocytes are found in the pulp in numerous experimental sections, whereas it is uncommon to find them in the other group of tissues. Klotz and Simpson [6] and others have referred to fatty degeneration of the endothelial cells of this organ, and in the experimental animals, fat is demonstrated in these cells lining blood spaces and in degenerated phagocytic endothelial cells in necrotic lymph follicles.

Kidney: The pathology in the kidney (Figs. 9 to 16) is the same in the two classes of specimens, with differences only of degree. Thus cloudy swelling and necrosis of tubular epithelial cells are found, but more of the former and less of the latter change is seen in the monkey kidneys. Numerous acidophilic degenerating cells are common to both groups of sections. Fatty degeneration occurs in the experimental as in the human tissues, both as to involvement of the tubular epithelium and as to the sparing of other structures. However, this type of degeneration is more pronounced in the animal specimens. Both classes of tissues show a consistent absence of inflammatory cells.

Congestion of small blood vessels, particularly of the glomerular tufts, is a regular feature of human cases, but is only occasionally found in monkey tissues. Hemorrhages are not seen in either group. Tubules contain much granular débris and casts of hyaline, granular

and calcareous types, more regularly, however, in human specimens. A type of degeneration of epithelial cells seems to be at least one source of "lime casts" in monkeys, such as Muller and Blaisdell [15] have described in human kidneys. Glomeruli are almost entirely unaltered, but their capsular spaces contain granular débris in specimens from man more than in those from the other group.

Heart: The heart (Figs. 17 and 18) in both sets of tissues shows fatty degeneration of finely granular form although, as in the liver and kidneys, fat is demonstrated as a rule in larger amounts in the rhesus specimens. Likewise, in both tissues, the muscle fibers are sometimes irregularly stained and cross-striations are indistinct, but these characteristics lose their significance when experimental sections are compared with those of control monkeys. Congestion and hemorrhage play a minor part in both human and monkey tissues and inflammatory cells are regularly absent.

Lungs: Sections of lungs (Figs. 19 and 20) show recent hemorrhages without inflammatory reaction, as a rule, however, more frequently and extensively in man. Similarly, congestion and edema are much more commonly found in the human tissues.

Stomach: The stomach has a greater tendency to congestion and hemorrhage in man, but there is the same type of small hemorrhages and extravasation of red blood cells without inflammatory reaction and without obvious lesions in the vessel wall.

Adrenals: Adrenal glands present contrasting pathology in that monkey sections often demonstrate necrosis accompanied by polymorphonuclear infiltration, which was found by us in one human case but is not recorded by others. Frequent congestion and an occasional hemorrhage are common to both classes of specimens.

Pancreas: The pancreas has been equally unaltered both in man and in monkey.

Lymph Nodes: Specimens of lymph nodes are not commonly available for study from human cases and little is recorded in the literature. In the rhesus, the changes are similar to those in the spleen.

Brain: The brain has been infrequently studied and shows little variation from the normal.

Summary

Fatty degeneration of the liver, kidney, heart and spleen is of the same type in man and rhesus, although more extreme in the latter.

Other degenerative changes of the liver, kidney and spleen are likewise similar as to incidence, type and location. Necrosis of the adrenal glands is only rarely seen in the human, although commonly in the rhesus.

In both sets of tissues, inflammatory cells are lacking in response to hemorrhage in any organ and to the degenerative changes in the kidney and heart. Polymorphonuclear and endothelial leucocytes are usually found associated with the liver lesions in monkeys, while seldom in man.

Hemorrhages and congestion tend to be more frequent and extensive in the liver, lungs and gastric mucosa in human cases, but the hemorrhages are alike in being focal, recent and without obvious lesions of the vessels.

Discussion and Conclusions

It should be borne in mind that these papers on the gross and microscopic pathology in the *Macacus rhesus* and comparison with human pathology are based on the use of one strain of yellow fever virus. However, the pathology induced by two other strains we are studying proves to be similar to that reported and discussed in these papers.

The lesions in the *Macacus rhesus*, brought about by experimental infection with yellow fever virus, seem to result from a severe intoxication, as in the case of human yellow fever and recently expressed by Klotz and Simpson.[6] Neither in the monkey nor in man is there any evidence of the localization of the virus. We have been unable to find either in human cases or in experimental tissues any constant bacterial form, or leptospiras or spirochetes demonstrable in Levaditi preparations.

The monkey specimens of the liver tend to confirm the fact that necrosis in the liver is essentially midzonal in type in yellow fever with less altered cells increasing toward the periphery of the involved region. Likewise, as in human cases, when the degenerative changes approach the limiting structures of the lobule, the most extreme necrosis is usually in the midzone.

Klotz and Simpson [6] have recorded that in the spleen there is a sequence of changes involving the lymph follicles, from early enlargement of the follicle due to hyperplasia of the endothelial elements, followed by loss of lymphocytes, to final degeneration of the endothelial cells. We have not observed in human or monkey tissues the first stage given; otherwise, a study of the rhesus sections makes it evident that the process described by these workers is probably correct. A stage of necrosis is obvious in the monkeys, but uncommonly seen in human spleens in which it is possible the stage might have been passed at the time of death. We would add, however, that in monkeys, degeneration and necrosis involves the lymphoid as well as the endothelial elements of the follicles.

The fatty degeneration of the heart muscle fibers and the same and other acute degenerative changes of the kidney in the rhesus monkey add to the evidence for the clinical manifestations of this disease in man.

If experience bears out the hope that the *M. rhesus* is regularly susceptible to the yellow fever virus, this animal will prove to be of incalculable value in the diagnosis of yellow fever in man because of the remarkably accurate reproduction of gross and microscopic lesions.

NOTE: In the photomicrographs, the lesions of yellow fever are compared as they occur in a human case and in rhesus monkeys. The human case is that of H. P., Accra, Gold Coast, diagnosed as yellow fever clinically and pathologically and from whom a strain of yellow fever virus was obtained by inoculation of a *M. rhesus* with the patient's blood. We are deeply indebted to Dr. D. Duff, Deputy Director, Medical and Sanitary Services, Dr. A. C. Paterson, Senior Medical Officer in charge of the European Hospital, and Dr. A. S. Burgess, Acting Director of the Medical Research Institute, Accra, Gold Coast, for records of this case and material for histologic study.

The sections were prepared from formalin-fixed tissues, stained with hematoxylin and eosin and, for the demonstration of fat, with scarlet red and hematoxylin.

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DESCRIPTION OF PLATES

PLATE 94

FIG. 1. H. P. Liver, showing irregular band of midzonal necrosis and mild diffuse hemorrhage. × 55.

FIG. 2. *M. rhesus*, No. 312. Liver, showing fringe of intact cells about central vein (left), beyond which is zone of necrosis infiltrated with inflammatory cells. Periportal cells (right) vacuolated. × 55.

FIG. 3. H. P. Frozen section of liver, demonstrating fat; less fat in necrotic midzone. × 40.

FIG. 4. *M. rhesus*, No. 312. Frozen section of liver, showing less fat in necrotic midzone. × 40.

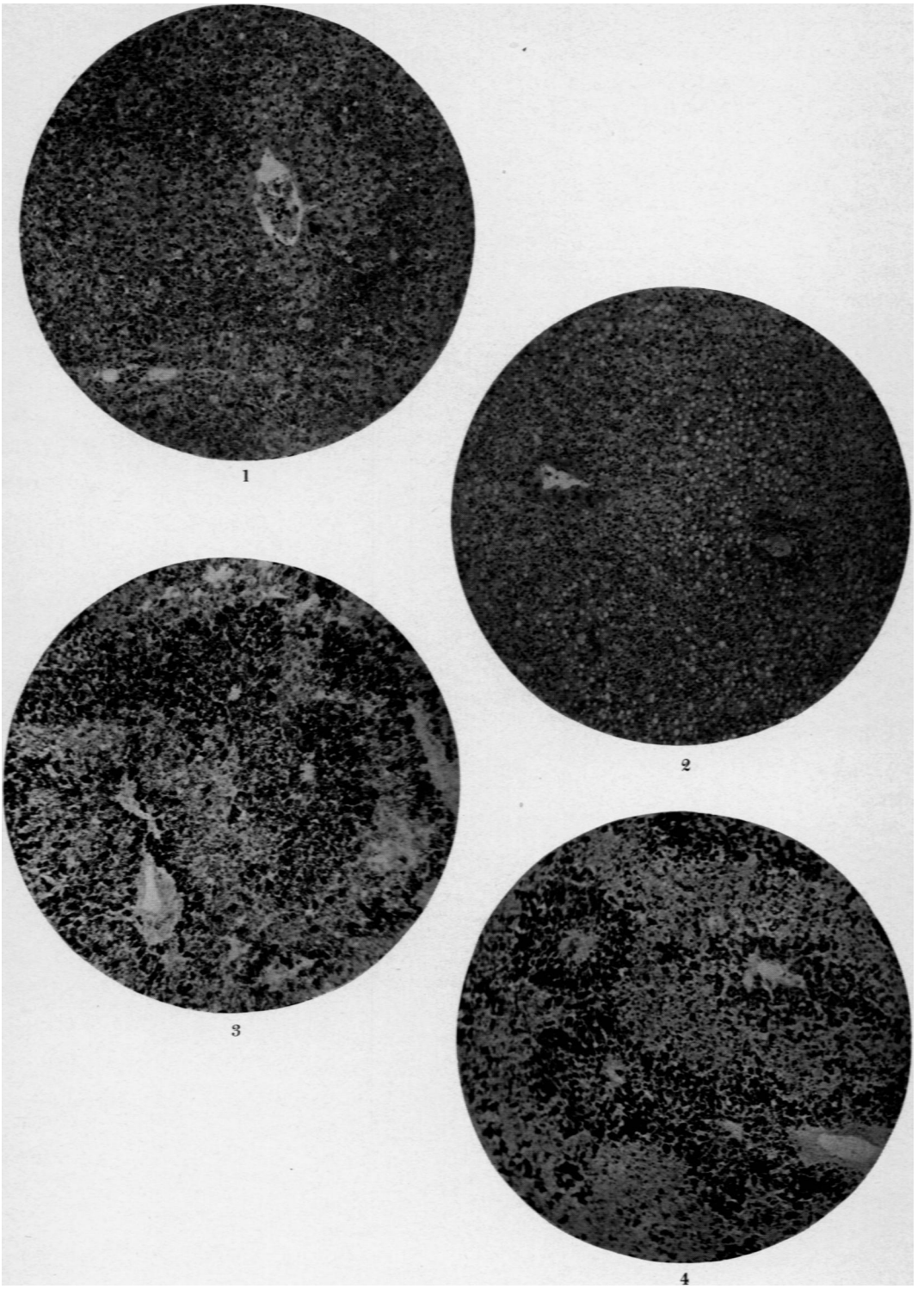

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PLATE 95

FIG. 5. H. P. Spleen, showing small lymph follicle surrounded by faint zone of enlarged endothelial cells, beyond which are congested blood spaces. × 85.

FIG. 6. *M. rhesus*, No. 344. Spleen, showing small lymph follicle surrounded by zone of enlarged endothelial cells, beyond which are congested blood spaces. × 85.

FIG. 7. Spleen of normal *M. rhesus*, No. 315. (Note magnification is lower than previous pictures of spleen.) × 45.

FIG. 8. *M. rhesus*, No. 362. Spleen. Necrosis in lymph follicle. × 150.

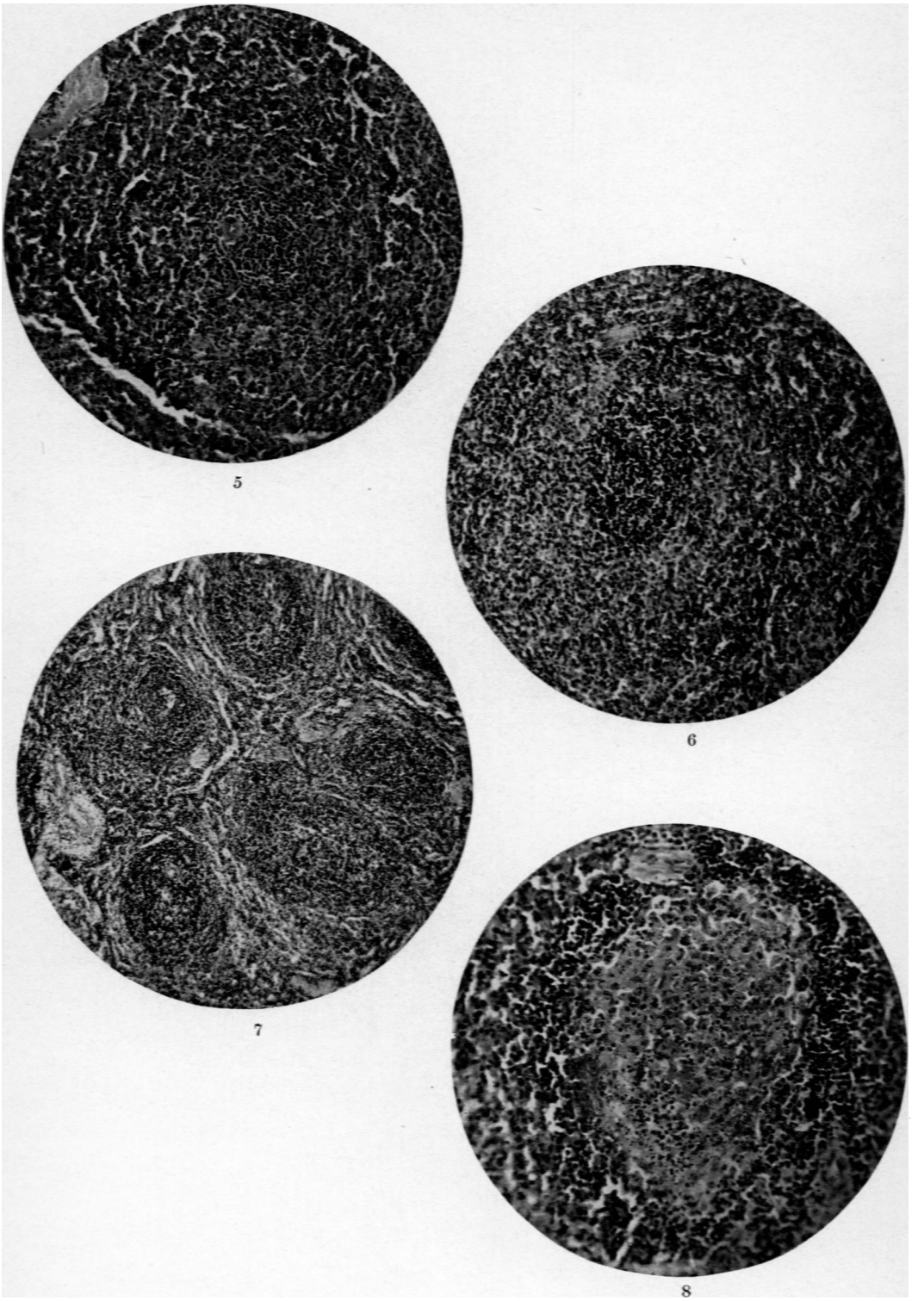

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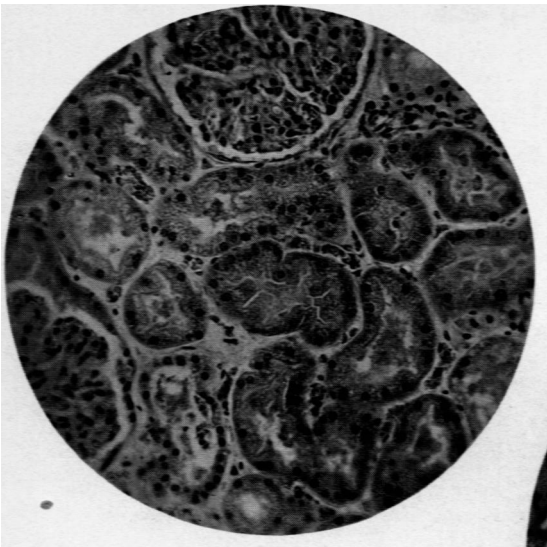
PLATE 96

FIG. 9. H. P. Kidney, illustrating swollen, acutely degenerated epithelial cells of tubules, and granular débris in lumina. × 150.

FIG. 10. *M. rhesus*, No. 327. Kidney, showing similar acute degenerative changes in vacuolated epithelial cells. × 150.

FIG. 11. H. P. Frozen section of kidney, indicating the presence of fat in tubular epithelial cells. × 150.

FIG. 12. *M. rhesus*, No. 327. Frozen section of kidney, demonstrating much fat in epithelial cells. × 150.



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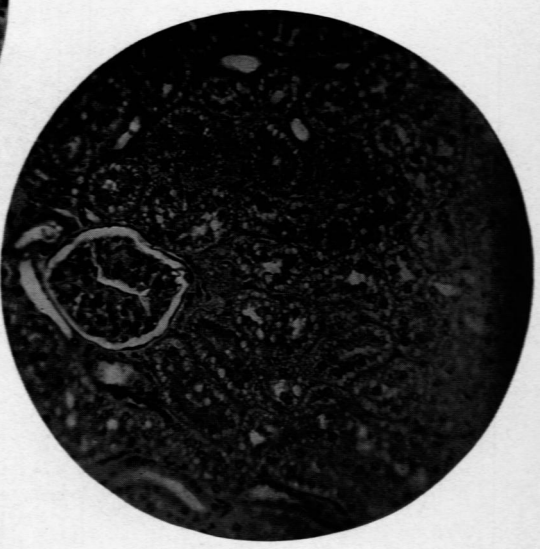

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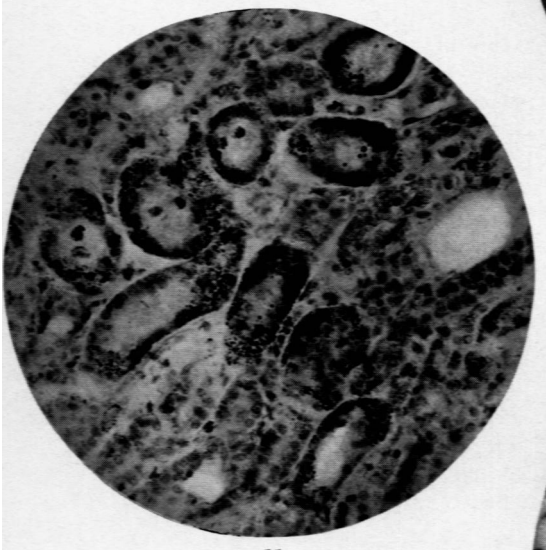

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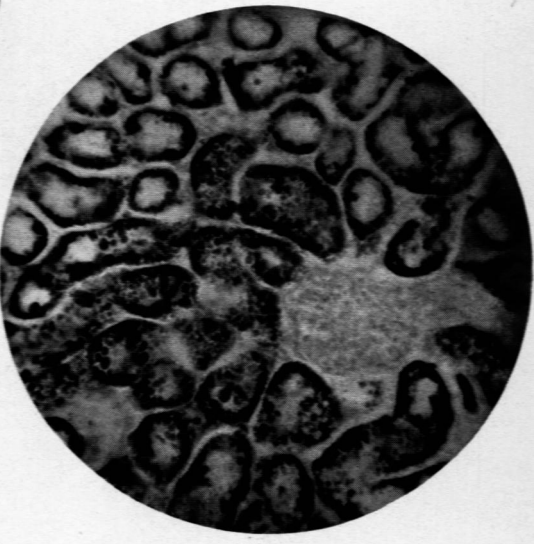

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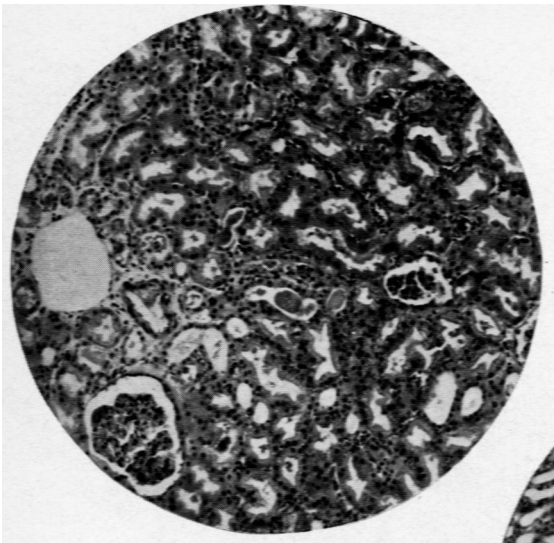
PLATE 97

FIG. 13. H. P. Kidney section, showing a few hyaline casts (center). Casts not as numerous in this case as is usually found in human kidneys. × 85.

FIG. 14. *M. rhesus*, No. 346. Kidney section, demonstrating several hyaline casts. × 85.

FIG. 15. H. P. Kidney, showing calcareous deposits ("lime casts") on either side of center of picture. × 150.

FIG. 16. *M. rhesus*, No. 253 A. Kidney, illustrating several clumps of calcareous deposits (about center), intensely stained by hematoxylin. × 150.



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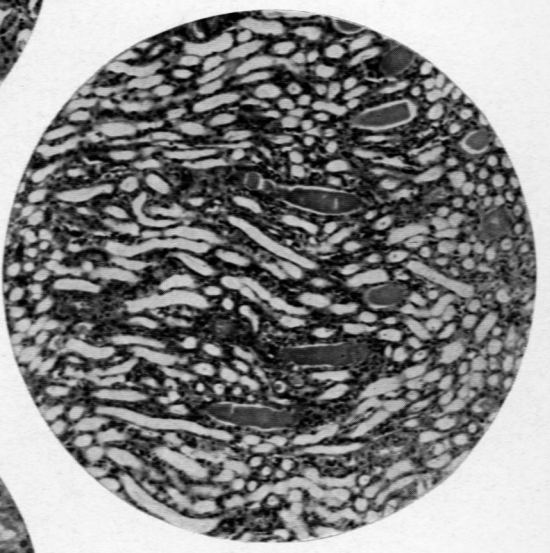

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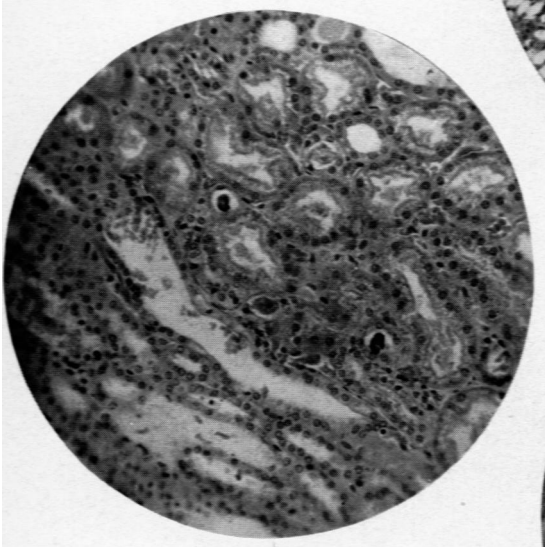

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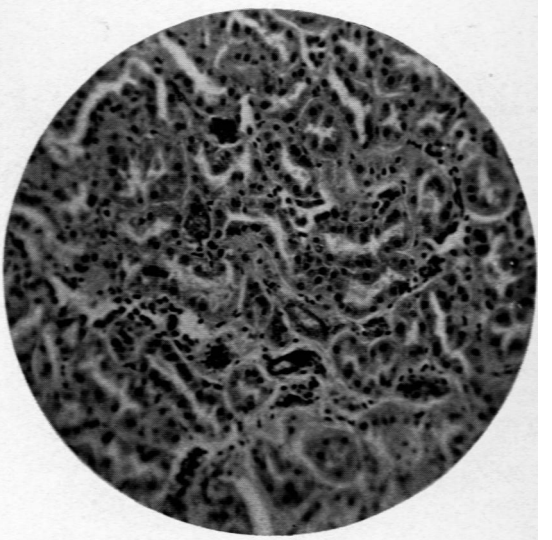

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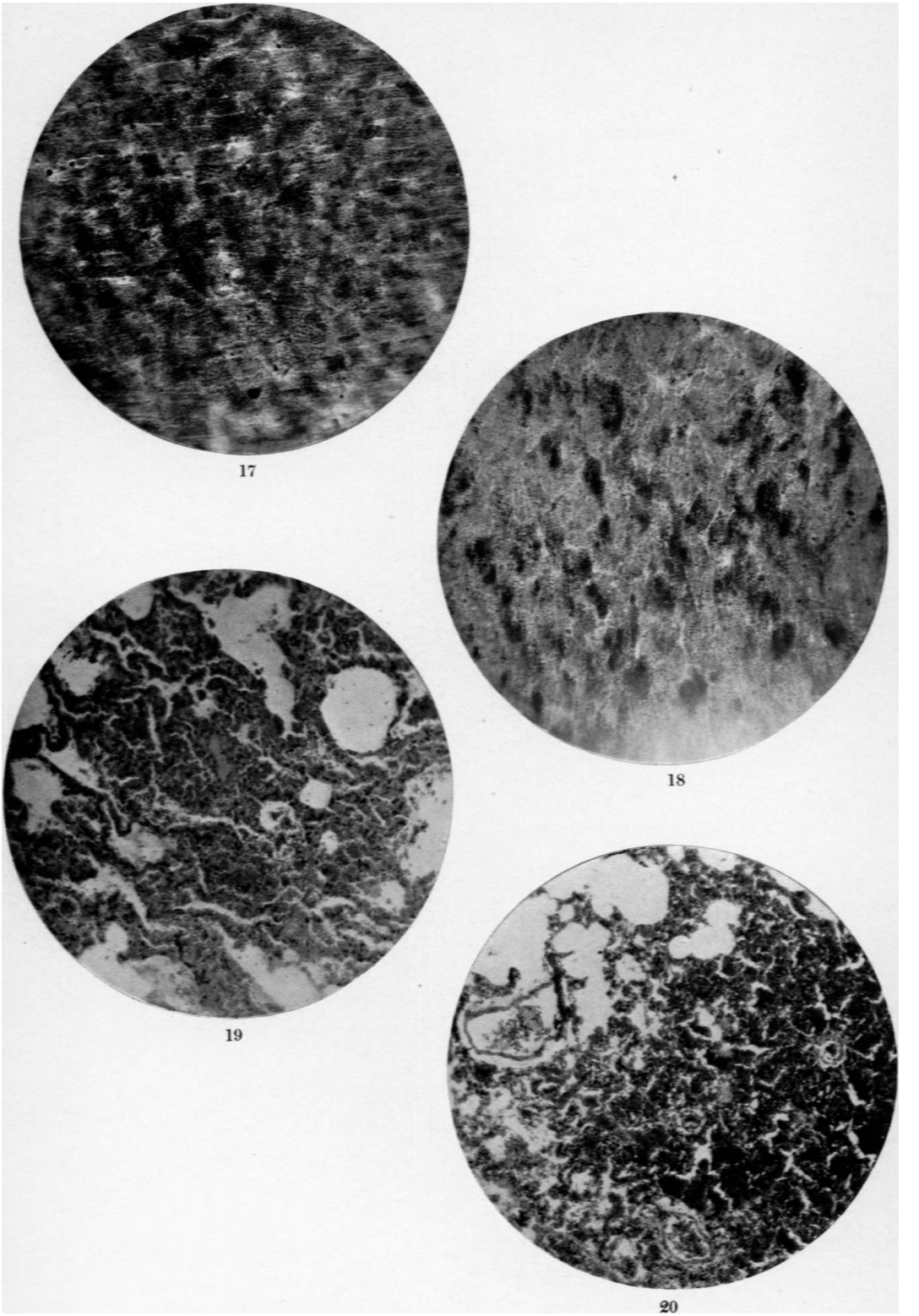
PLATE 98

FIG. 17. H. P. Frozen section of heart, demonstrating much finely granular fat in muscle fibers. × 150.

FIG. 18. *M. rhesus*, No. 327. Frozen section of heart, showing finely divided fat irregularly distributed among muscle fibers. × 150.

FIG. 19. H. P. Section of lung, showing small area of recent hemorrhage into alveolar spaces. × 85.

FIG. 20. *M. rhesus*, No. 316. Lung section, showing a similar recent hemorrhage into alveolar spaces. × 85.



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