# JAUNDICE IN MICE DUE TO ANOMALIES OF THE BILIARY TRACT

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Plates 2 to 6

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During the past two years a disease characterized by unthriftiness, jaundice, and stunted development has been endemic in the young mice of the breeding stock maintained by one of us for commercial purposes. Although the incidence has remained low (approximately 2 per cent) and although no infectious etiological agent has been demonstrated, the rather remarkable pathological lesions associated with anomalies in the development of the biliary tract and their possible bearing on similar human instances, have led us to give a detailed description of the condition.

## *General Facts Regarding the Breeding Colony*

The colony was initiated in 1949 with the CFW strain of mice obtained from Mr. C. N. Wentworth Cumming of Carworth Farms, New City, New York. These mice have been propagated by random mating since that date without the introduction of new breeders. The output of CFW mice has averaged 5,000 animals per week. For a short period during 1951 to 1952 a small number of $C_3H$ mice were raised as well; no disease was noted in these animals. No other mice and no rats have been raised.

The colony of CFW mice has been maintained in a detached rodent-proof building. Each breeding box has contained one male and six females. The diet has consisted of commercial "lab blocks" furnished by Allied Mills; no dietary supplements have been employed. Water has been available at all times. The bedding has consisted of white pine shavings. At 6 weeks of age all prospective breeders have received one injection of a trivalent vaccine composed of *Salmonella typhimurium*, *Salmonella enteritidis*, and a strain of *Escherichia coli*[1] isolated at Carworth Farms from the original breeding stock. Each injection has consisted of $5 \times 10^9$ organisms killed by exposure to ultraviolet light. Although routine cultures have not been taken regularly there has been no gross evidence suggesting the presence of mouse typhoid in the colony. Although the colony has remained free of the "chatters," the breeders have been immunized for the past year with a commerical formalin-inactivated vaccine made from *Pasteurella multocida*.[2] Epidemic diarrheal disease has been noted from time to time among the suckling mice; this appears to be similar to the disease described by Cheever, Mueller,

[1] Prepared by Dr. A. S. Schlingman of Parke, Davis and Company.

[2] Manufactured by Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

Pappenheimer, and Enders (1(*a*)-(*d*)) and by Runner and Palm. (2) Quite characteristically the suckling mice have been affected almost entirely during the autumn and winter months; during the warmer period of the year the amount of diarrheal disease has been nearly nil.

The first signs of the hepatic disease usually appear during the 3rd week of life. Unthriftiness, a bleached out appearance, and the development of a potbelly are the first manifestations noted. Usually within a week jaundice appears. The disease tends to pursue a chronic course; the rate of growth is almost always retarded and the relatively few mice that survive indefinitely are usually stunted in appearance. By the end of 3 months the cumulative mortality rate among jaundiced mice reaches 90 per cent. Attempts to breed the relatively few survivors have met with failure.

The over-all incidence rate of jaundice in the colony during the past two years has been low, averaging in the neighborhood of 2 per cent. It has been rare to find more than one jaundiced mouse in a given breeding box at one time. As in the case of the diarrheal disease, the hepatic disease has been more prevalent during the fall and winter months; furthermore, as in the case of the diarrheal disease, it has remained largely limited to first, second, and third litters. Only very rarely has the disease been observed in fourth or later litters. Although there has been some tendency for the hepatic disease to occur in mice suffering from epidemic diarrhea the association has not been a complete one. The majority of mice suffering from diarrhea do not develop jaundice, whereas jaundice occasionally develops in mice apparently free of the diarrheal disease. There has been no evidence of spread from one mouse to another within the breeding colony.

## *Laboratory Studies*

Dissection and examination of the common duct failed to reveal any obvious cause of the biliary obstruction. No pathogenic ova were recognized in smears of the intestinal contents. Fecal and intestinal content studies were negative for the presence of members of the *Salmonella* group. Bile aspirated from the gall bladder showed no growth when cultured aerobically or anaerobically. Cultures taken from the necrotic liver lesions were usually sterile although occasionally strains of *Proteus* or coliform organisms were isolated. Dark field examinations of liver emulsions were negative for leptospira as were rabbit serum–saline cultures. Two guinea pigs inoculated intraperitoneally with a suspension of ground hepatic cells derived from the pooled livers of several jaundiced mice remained well for 30 days; when sacrificed and autopsied at this time no gross or microscopic lesions were recognized. 3 to 4 week old mice inoculated intraperitoneally or intracerebrally with hepatic cell suspensions derived from the livers of jaundiced mice remained well throughout a 3 week period of observation; no gross lesions or microscopic lesions were recognized at autopsy. On several occasions one or two jaundiced mice were placed in a breeding box with several normal mice (CFW and Tumblebrook strains) of the same age and kept there until death occurred. The normal animals were held under observation for 2 to 3 months after the initial exposure; in no case were signs of illness observed and at autopsy no gross lesions were recognized.

## *Pathology*

*Gross Findings:*

At autopsy the chief findings recognizable with the naked eye are stunted development, bile-stained tissues, markedly dilated gall bladder and biliary tree, and circumscribed areas

of necrosis in the liver. Ascites is minimal or absent. The distention of the common duct appears to start a short distance above the ampulla of Vater. However, distention of gall bladder and ducts is not always noted. In our latest series of 17 jaundiced mice, all showing biliary cirrhosis microscopically, 5 had no gross dilatation of the gall bladder or common duct; in 3 others, only the gall bladder appeared dilated. The liver is usually tawny but not hobnailed. Gross areas of necrosis are common findings in the liver of young mice; these lesions are met with but rarely in older mice, and the occurrence of pitted areas suggests that healing has taken place. The spleen is frequently enlarged but no gross lesions have been recognized. The other organs show no gross abnormalities.

*Histopathology:*

The material studied consisted of Bouin-or formalin-fixed pieces of liver, gall bladder, stomach, spleen, pancreas, kidney, and intestine from jaundiced mice of various ages. Representative blocks were embedded in paraffin and stained with hematoxylin-eosin, Weigert hematoxylin–van Gieson, Giemsa, Masson's trichrome stain, and Ziehl-Neelsen carbolfuchsin. A few livers were examined for leptospira, using Levaditi and Warthin-Starry impregnation. In 6 mice, the entire liver, biliary tract, duodenum, and head of pancreas were sectioned serially at 6 $\mu$.

*Extrahepatic Ducts and Gall Bladder.*—Marked dilatation of the gall bladder and ducts, though not invariable, was present in the majority of cases. In the cases with dilatation, serial sections disclosed that there was complete absence of a segment of common duct near the duodenum, so that the blind end was separated by pancreatic tissue from the duodenum. (Fig. 2). The pancreatic duct in every case serially studied could be traced through the duodenal wall and opened normally into the duodenal lumen. On the other hand, in a jaundiced mouse in which there was neither gross nor microscopic distention of the biliary tract, the common duct was patent throughout, joining the pancreatic duct just before penetrating the duodenal wall. It was perhaps somewhat narrowed before dividing into cystic and hepatic ducts, but was definitely not atretic.

In the mice showing great distention of the biliary tract, it was difficult to distinguish common, cystic, and hepatic ducts in the sections, since all were cystically dilated, with many valve-like infoldings of the mucosa. What could be definitely established was that the hepatic ducts ended in blind culs-de-sac shortly after reaching the liver, and did not branch into small intrahepatic ducts (Fig. 4). These terminal saccules were lined with flattened cells, and distended with inspissated, bluish staining mucoid material. In one mouse, it was noted that the liver tissue adjacent to these terminal saccules was flooded with mucoid material, most of which had been ingested by phagocytes (Kupffer cells), the nuclei of which formed flattened crescents at the periphery of the mucoid masses. It would appear that some of the terminal saccules had ruptured with extravasation of their contents. The distended bile ducts and bladder were lined throughout by low cuboidal epithelium. The wall contained some smooth muscle, but was composed chiefly of dense fibrous tissue.

In a small number of mice, not including any of those serially sectioned, secondary infection of the distended biliary passages had occurred (Fig. 1). In these cases, there was acute or chronic ulceration of the lining mucosa (Fig. 3), inflammatory thickening of the walls of gall bladder and ducts, and much exudate—purulent or hemorrhagic—both inside and on the peritoneal surfaces. Sometimes the entire lumen was filled with a solid mass of necrotic exudate. Gram-negative bacilli could be demonstrated in the exudate of one such case.

In sections stained by the Ziehl-Neelsen method, phagocytes filled with red-staining pigment were abundantly present upon the surface of the ulcers, in the thickened walls of the ducts, and in the portal spaces of the liver. In Giemsa preparations, the pigment took a greenish blue color, and it was strongly fluorescent. It thus conformed to the character of

ceroid pigment. A small amount of such pigment was found in the smooth muscle cells of the duodenum.

*Intrahepatic Lesions.*—There were present in every animal examined, although with some variation in intensity and distribution, lesions which may be discussed under the following categories: (1) absence of normal intrahepatic bile ducts, (2) biliary cirrhosis, and (3) areas of necrosis.

A careful review of our material and comparison with control material establish the fact that there is complete or almost complete absence of normal biliary ducts in the portal spaces. This must be regarded as a significant and cardinal feature of this disease. It explains why jaundice occurred in one mouse (No. 6803) in which there was no complete obstruction of the extrahepatic biliary passages.

Biliary cirrhosis of greater or lesser degree was found in all the livers examined. The portal spaces were surrounded by a mass of epithelium-like cells with pale nuclei, accompanied by a sparse number of lymphocytes, rare polymorphonuclears, and possibly some fibroblasts. The collagenous stroma, as shown in van Gieson and Masson preparations, was increased. The epithelia sometimes formed cords of double cells, and often became differentiated into small ductules. These could readily be distinguished from normal ducts by the pallor of their nuclei, and by the fact that the long axes of the nuclei were circumferentially disposed, instead of being oriented perpendicular to the basement membrane. Indeed, no basement membrane could be demonstrated about these new formed ductules (Fig. 5).

Areas of necrosis were found in about 80 per cent of the animals. They were often very large, occupying an entire low power field, and had the appearance of anemic infarcts (Figs. 6 and 7). In some, evidently recent in origin, only the liver cells were necrotic, and the sinus endothelium still preserved its staining. In others, all components showed complete loss of nuclear staining. Sometimes there was slight polymorphonuclear leucocytic reaction in and about the necrotic area. Various stages of healing were encountered from early invasion of fibroblasts or endothelial cells, to complete replacement with young connective tissue. In some of the necrotic foci, the cells appeared to be undergoing liquefaction. In sections prepared with Weigert's elastic tissue stain, only a rare hepatic arteriole could be found. In an occasional arteriole the lumen was almost obliterated, and the muscular wall replaced by connective tissue.

Despite the obvious obstruction of the extrahepatic biliary passages, and the intense jaundice, there was no distention of the bile canaliculi with pigment plugs, nor was there bile pigment in the Kupffer cells. The explanation for this is not apparent.[3]

*Stomach.*—Sections of the stomach from 30 mice were available. The epithelium of both squamous and glandular portions was intact, and free from inflammatory changes. The cavity, in addition to much undigested cellulose, contained many bacteria, chiefly Gram-positive bacilli. In mouse 5320, there were many yeast cells on the surface of the epithelium; some budding forms were seen, but there was no mycelial formation.

In 3 out of the 30 mice, protozoan bodies in great numbers were found free in the gastric tubules. These were unquestionably identical with the *Cryptosporidium muris*, discovered and described by Tyzzer in 1910 (4). All the characteristic features pictured in this paper were readily seen in Giemsa-stained preparations of our material (Fig. 9). As Tyzzer had first pointed out, the parasites were extracellular, but many of them were attached to the

[3] Ogata (3), in his study of the production of biliary cirrhosis in laboratory animals by the ligation of the common duct, makes the following interesting statement (p. 311): "It is important that... bile plugs may be completely absent in some experimental animals, for example, *small rodents*." His experiments were performed on rats, not mice. He gives no explanation for this.

epithelial cells by a filamentous organ of attachment. They gave rise to no inflammatory reaction.

In view of the familiar biliary and hepatic lesions induced in rabbits by another species of coccidium, *Eimeria stiedae*, a careful but unsuccessful search was made for cryptosporidia in the duodenum, gall bladder, biliary ducts, and liver of the jaundiced mice. They were not found, and it is obvious that this interesting infestation, present in only a few of our animals, played no role in the pathogenesis of this disease.

In the case of all mice in which the stomach was examined, protozoan-like bodies were discovered in the pars pylorica of the gastric mucosa (Fig. 10). They lay in large vacuoles within the cytoplasm of the epithelial cells. Although present at all depths of the mucosa, they were most numerous in the superficial portion from which they were desquamated into the lumen. They were not present in the cells of the duodenal mucosa, but were sometimes found in great numbers within the lumen of the duodenum. They varied greatly in size and structure. Some contained a single, small, deeply staining eccentric spherical nucleus. Others had another crescentic chromatin mass. In still other forms, the chromatin was dispersed in a number of discrete small, deeply staining masses. The cytoplasm often contained eosin-staining globules, which could not be identified as ingested red cells. There was a distinct capsular membrane. In the absence of all inflammatory reaction, it seems extremely unlikely that these structures were merely mononuclear leucocytes in various stages of degeneration. Although we are uncertain as to their nature, it is our belief that they represent protozoan-like parasites, an opinion concurred in by the parasitologists who have seen these preparations.

*Pancreas.*—There were no significant changes, and the intrapancreatic ducts were not dilated.

*Other Viscera.*—No significant lesions were discovered in the spleen, kidneys, or intestines. The esophagus of one mouse presented a double lumen separated by collagen and enveloped in a single muscular coat (Fig. 8).

## DISCUSSION

A clue to the pathogenesis of this interesting disease was obtained only when serial sections of the entire biliary tract were studied. It had been assumed, because of the striking dilatation of the bile ducts and gall bladder, and the biliary type of cirrhosis seen in the liver, that there must be some obstructive lesion of the common duct. But it was difficult to demonstrate this in the gross. Serial sections proved conclusively that the obstruction in some cases at least was due to congenital absence of the segment of common duct entering the duodenum. The pancreatic duct entered normally, but the common duct ended blindly at a distance from the duodenum. There was dilatation of the biliary tract beyond this point. The contents, so far as one can judge from the sections, were some sort of mucoid coagulum.

The hepatic ducts appeared to penetrate the liver for only a short distance and to end in culs-de-sac, without branching into small ducts. This was true at least of the serially studied specimens.

The hepatic lesions varied from case to case. Most of the livers showed a definite biliary cirrhosis. There was multiplication of epithelial cells about the portal spaces. In some cases these differentiated into distinct ductules, giving

the tissue a spongy appearance. In others, they grew out as solid sprouts of cells without luminal formation. They were sometimes accompanied by a sparse number of lymphocytes, plasma cells, and some polymorphonuclears. In a few of the livers, biliary cirrhosis and proliferation of bile duct epithelium were virtually absent, and the only abnormality was a moderate increase in collagen about the portal spaces.

Biliary cirrhosis of greater or less severity was found in 88 per cent of the 36 livers examined. Although new formed ductules, lined by cells with pale nuclei often ranged with their long axes parallel to the lumen, were frequent, *normal bile ducts* with a distinct basement membrane and closely apposed nuclei perpendicular to the lumen were not seen. Only exceptionally, there were a few ducts in the region where the extrahepatic ducts penetrated the liver for a short distance. In general, one may say that there had been complete failure of the intrahepatic bile ducts to undergo normal differentiation. This was true even in cases in which no complete obstruction of the extrahepatic bile ducts could be demonstrated.

A second equally characteristic lesion was the occurrence of circumscribed areas of necrosis. These varied greatly in number and extent, and were not directly correlated with the biliary cirrhosis. Thus, in mouse 6776, one of those serially sectioned, they were extremely large, occupying almost half of the liver parenchyma; in this animal, there was virtually no biliary cirrhosis. In other mice, they were absent, in spite of extensive cirrhotic lesions (mouse 5616). In our entire series of 36 mice, they were found in 28, or 77 per cent.

The explanation for these necroses is still to be discovered. They are not associated with secondary infection. Thus in serially cut mice 6776, 6803, 6804, and 6812, in which they were particularly massive, there was complete absence of inflammatory changes in the distended biliary passages. The light leucocytic infiltration about some of the necrotic foci was probably a reaction to the necrotic tissue itself. The infarct-like character of the lesions suggests that there may have been a defective arteriolization, and indeed in these particular animals, we have been unable to find arterioles in the portal spaces. Convincing proof of this hypothesis will require further observation, using injection techniques. The possibility that the necroses are due to extravasation of bile—the usual explanation in human cases of biliary obstruction with necroses—is unlikely; no bile pigment is seen in the necrotic areas.

In several mice, distention of the biliary tract was accompanied by definite evidence of bacterial infection. There was hemorrhagic and purulent exudate within and about the distended bile passages, mucosal ulcerations and local peritonitis, and pericholangitis. It is obvious that these inflammatory lesions were secondary ones since they were absent in most of the mice. We feel equally certain that the *Cryptosporidium muris* parasites which were present in the gastric mucosa of a few of the mice played no part in the pathogenesis of the disease.

In short then, we are dealing with chronic biliary obstruction due to the absence of a segment of the common bile duct or of the intrahepatic ducts or a combination of the two. That there should be anatomic variations as regards the site of obstruction and the character and severity of the hepatic changes, is to be expected. In the few completely studied animals, there was complete absence of the common duct in the segment proximal to the duodenum, as well as absence of the small intrahepatic ducts, but further studies may well show variations from this pattern.

An entirely comparable disease is of not infrequent occurrence in infants. As far back as 1916, Holmes (5) was able to collect over 100 cases of congenital obliteration of the bile ducts, and his classic paper includes diagrammatic sketches of the variety of abnormalities found in the reported cases. The condition found in our serially sectioned mice corresponds most closely to the case reported by Legg (6), the more common abnormality in humans being the atresia at one or more points of a portion of the biliary tract, which is represented by a fibrous cord. Dahl-Iverson and Gormsen (7) in 1943, assembled 264 cases. As in our mice, icterus is not necessarily present at birth, and indeed its appearance may be delayed as long as 5 weeks (8). The explanation for this delay, given by Ylppo (9), is that the liver of the newborn has a capacity for storing a certain amount of bile pigment, and until this is overcome, the blood concentration does not rise to the level necessary to produce cutaneous pigmentation.

The complete absence of intrahepatic biliary ducts also has its analogue in human pathology. Ahrens, Harris, and MacMahon (10) have recently collected 10 such cases from the literature and reported 4 additional cases, in which, in careful study of liver sections, the portal spaces appeared to contain no ductules. In some of these cases there were associated anomalies of the extrahepatic ducts. The authors discuss the difficulty of reconciling the absence of intrahepatic ducts with current embryologic theory since the hepatic parenchyma is supposed to be derived from biliary epithelium. It has been suggested, however, that bile ducts and hepatic cells arise from separate anlagen in the hepatic diverticula from the foregut. Although this view has not met with general acceptance, it would seem to fit in with our observations and those in the human cases, that normal liver parenchyma may develop in the absence of intrahepatic bile ducts. Bremer (11) suggests that the liver cells may become differentiated from the bile duct epithelium before canalization has occurred, and that subsequent duct formation may not take place.

As regards the pathogenesis of the disease in mice, we have entertained three possibilities. (1) The lesions may be true genetic malformations. If this be the case, the responsible gene(s) must be a relatively rare one since the incidence of recognizable lesions is so low. Furthermore, the marked seasonal occurrence would suggest that non-genetic factors played a role in the pathogenesis of the lesions as well. (2) The malformations may be due to infection of the mother with an unknown agent during gestation. We have no evidence for or against this possibility, which comes to mind only because of the now well recognized malformations associated with the occurrence of rubella in early pregnancy. (3) The lesions may be the result of a protozoan infection

acquired early during fetal life. For this possibility too, there is as yet no substantial evidence. It is, however, interesting that all the diseased mice hitherto examined in which the pyloric region could be studied, have been found to harbor protozoan-like bodies. Similar bodies, though mixed with cellular detritus, have been found free in the duodenal lumen and within the distended bile passages. The possible relations of these still unidentified parasites to the disease can only be determined by further study.

There are, in the literature, some interesting observations which indicate that distention of the biliary passages in mice may occur in the absence of organic stenosis or atresia of the ducts. Furth, Gadsen, and Upton (12) have described hyperplasia and cystic dilatation of the extrahepatic biliary ducts in mice bearing grafts of pituitary tumors which secrete thyroid-stimulating hormones. They suggest that this change in the biliary tract is related to some as yet unidentified pituitary hormone. Unlike our mice, these animals showed no hepatic lesions (13), which is difficult to understand, even if the distention was due to long standing functional stenosis. Hypertrophy of the biliary ducts in mice following prolonged injections of various estrogens has been described by Gardner, Allen, and Smith (14). Histologically, the thickening was found to result from glandular hyperplasia. The gall bladder was not distended; no mention is made of jaundice or of biliary cirrhosis, so that we may infer that these changes brought about by endocrine factors did not lead to organic obstruction. Obviously, these conditions have no bearing upon the disease with which we are concerned, in which there is no reason to suspect endocrine factors in the pathogenesis.

## SUMMARY

There has been described a previously unrecognized disease of mice, characterized by progressive jaundice, first appearing during the nursing period. This has been shown to be due to congenital absence of the terminal segment of the common bile duct, or to the absence of intrahepatic ducts. In the former case, there is distension of the cystic and hepatic ducts, and of the gall bladder, with mucoid material. Biliary cirrhosis and infarct-like areas of necrosis are commonly found in the liver. The cause of the necroses has not been positively determined, but it is suggested that they result from defective arteriolization of the hepatic parenchyma. Inflammatory lesions of the biliary passages, when present, are attributed to secondary bacterial infection. Protozoan-like parasites were present in the gastric epithelium of all mice examined. Their relationship to the biliary and hepatic lesions is as yet undetermined.

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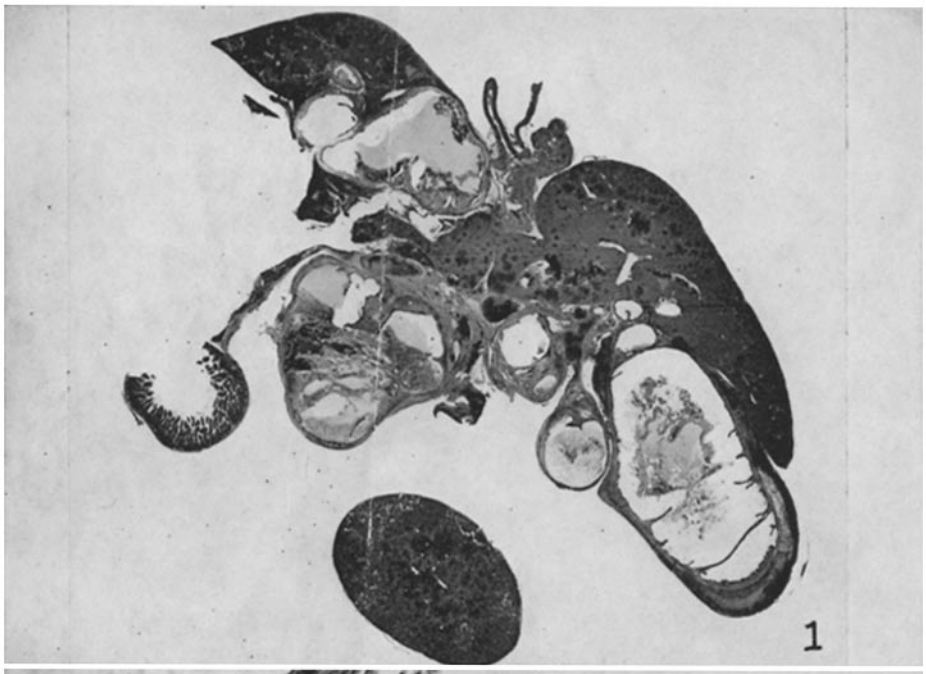
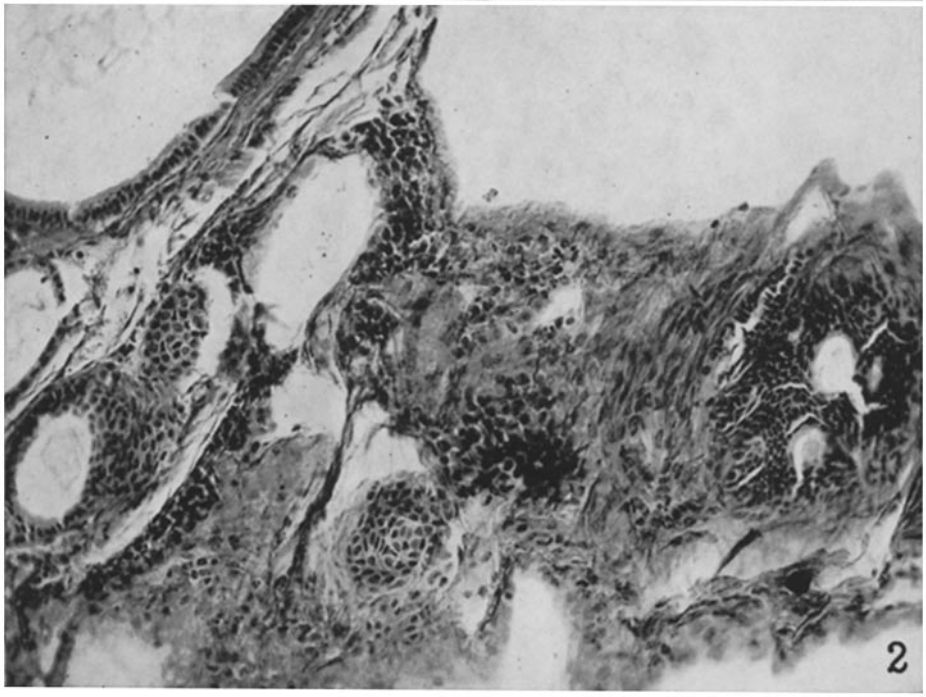
## EXPLANATION OF PLATES

### PLATE 2

FIG. 1. Mouse 5224. Low power view of distended gall bladder and biliary passages. In this animal there was secondary infection; purulent and hemorrhagic exudate is found within the dilated ducts and gall bladder. Hematoxylin-eosin. × 8.

FIG. 2. Mouse 6812. Section near blind ending of common duct. There are solid or partly canalized nests of epithelium surrounded by fibrous or muscular tissue loosely infiltrated with polymorphonuclear leucocytes. Weigert hematoxylin–van Gieson. × 225.



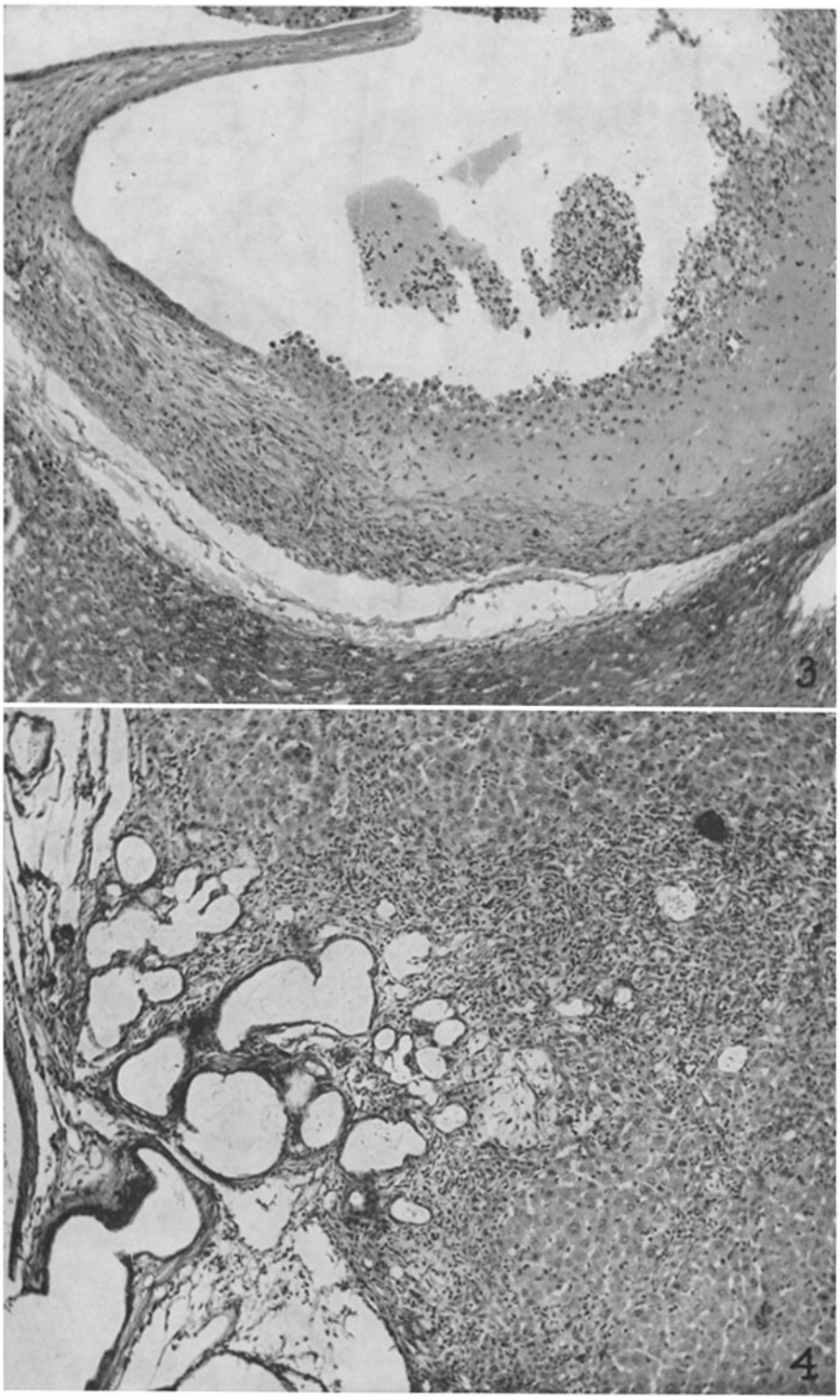




(Pappenheimer *et al.*: Jaundice in mice due to biliary tract anomalies)

## PLATE 3

FIG. 3. Mouse 5224. Greatly dilated cystic duct. The mucosa is ulcerated, and there is purulent exudate in lumen. Hematoxylin-eosin. × 110.

FIG. 4. Mouse 6812. The extrahepatic duct ends in blind saccules which extend only a short distance into hepatic parenchyma. Weigert hematoxylin–van Gieson. × 95.

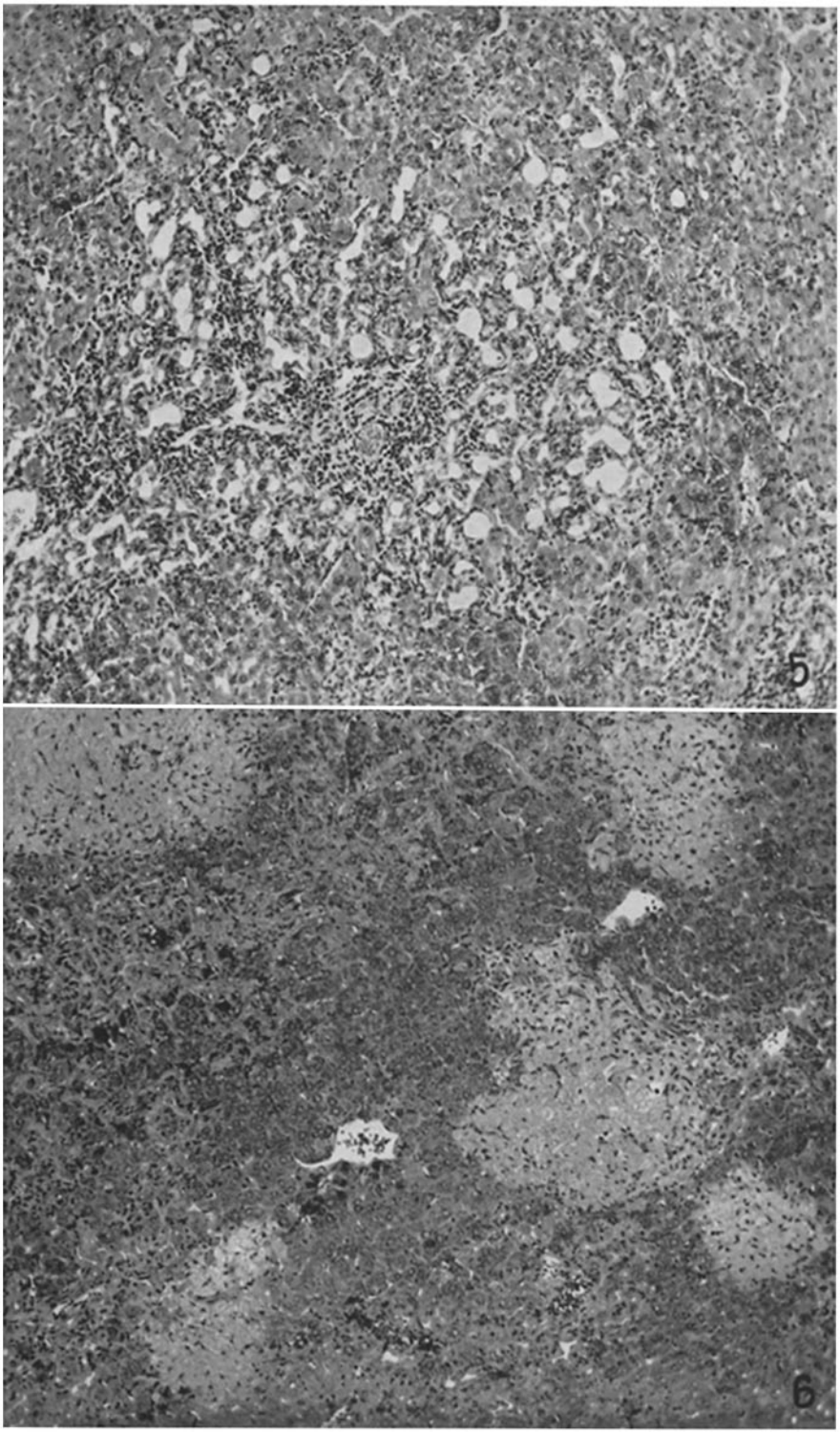



(Pappenheimer *et al.*: Jaundice in mice due to biliary tract anomalies)

## PLATE 4

FIG. 5. Mouse 5320. Extensive biliary cirrhosis, with proliferation of bile duct epithelium to form new ductules. There are no normal bile ducts in the portal spaces. Hematoxylin-eosin, × 145.

FIG. 6. Mouse 5224. Liver showing numerous focal necroses. Hematoxylin-eosin. × 140.

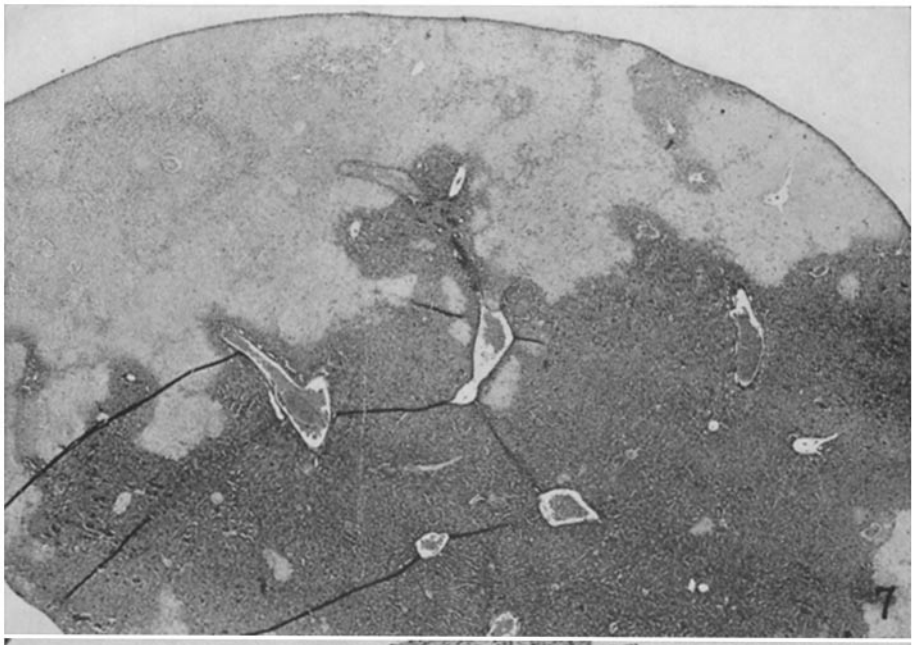


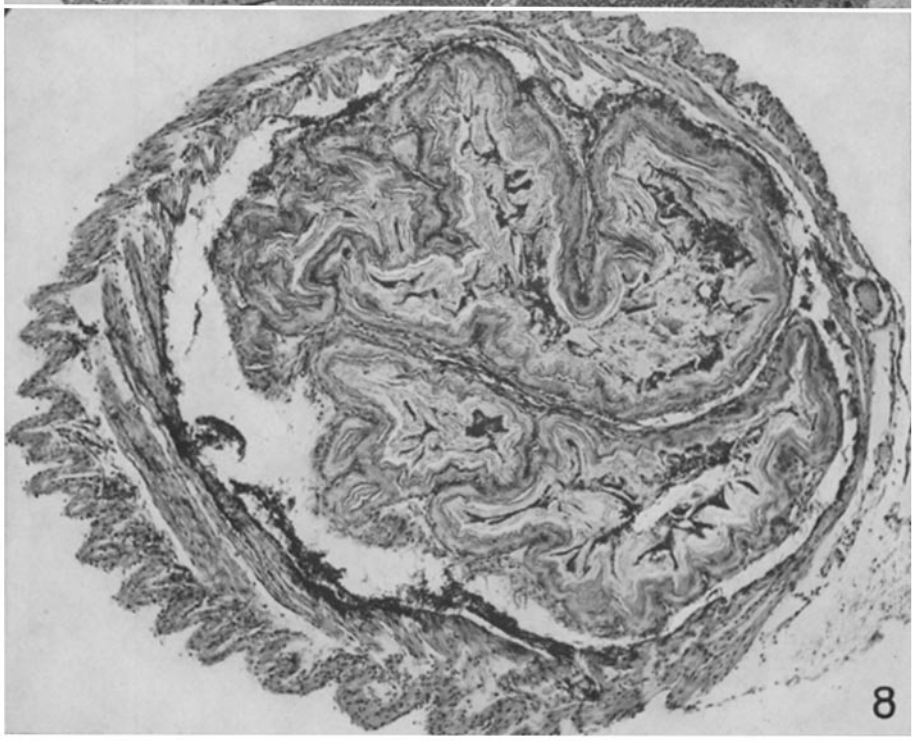

(Pappenheimer *et al.*: Jaundice in mice due to biliary tract anomalies)

PLATE 5

FIG. 7. Mouse 6812. Massive infarct-like areas of necrosis in liver. Note absence of normal bile ducts and hepatic arteries in portal spaces. (Black lines are folds in section.) Weigert hematoxylin–van Gieson. × 28.

FIG. 8. Mouse 6812. Esophagus, showing a double lumen separated by collagen, and enveloped in a single muscular coat. The heavily keratinized epithelium is surmounted by colonies of bacteria. Weigert hematoxylin–van Gieson. × 80.



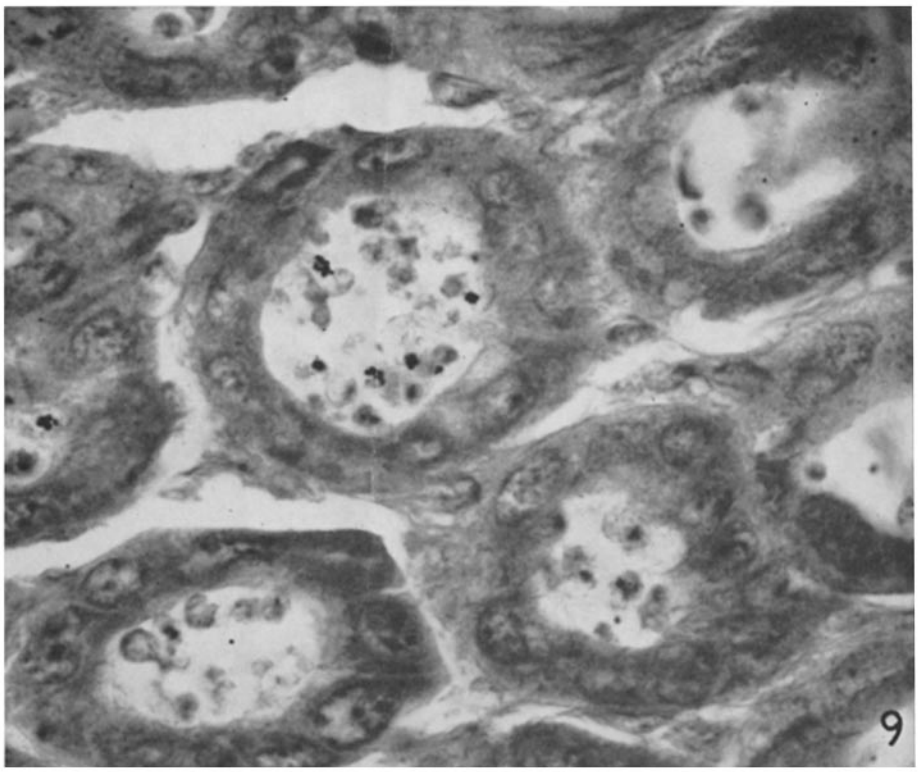



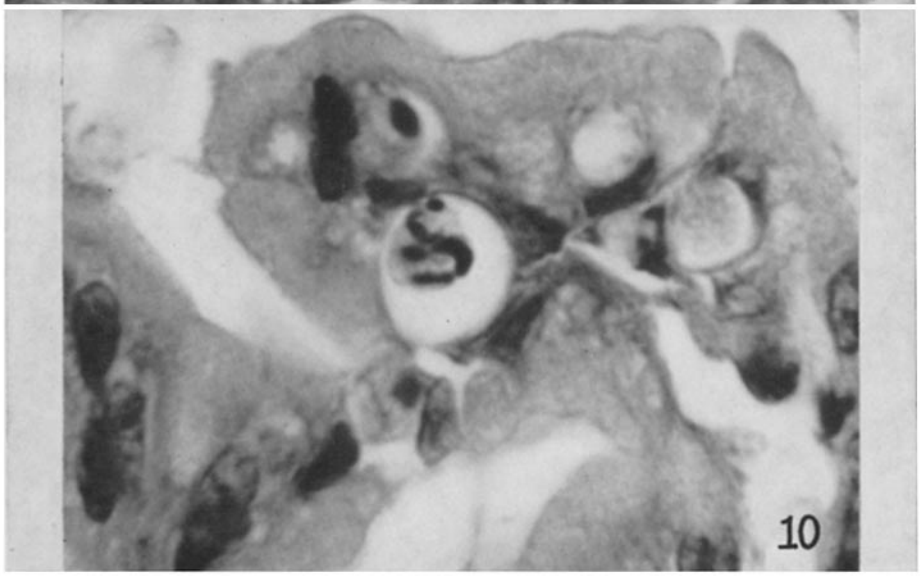

(Pappenheimer *et al.*: Jaundice in mice due to biliary tract anomalies)

PLATE 6

FIG. 9. Mouse 5469. *Cryptosporidium muris* in gastric tubules. Giemsa. × 1140.

FIG. 10. Mouse 6803. Protozoan-like bodies in the epithelial cells of the pars pylorica of the stomach. Weigert hematoxylin–van Gieson. × 1460




(Pappenheimer *et al.*: Jaundice in mice due to biliary tract anomalies)