BILIARY CIRRHOSIS IN THE RABBIT.*

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The statement concerning biliary cirrhosis which is commonly found in text-books on pathology is to the effect that obstruction of the bile-duct without infection does not lead to cirrhosis of the liver. This is expressed by Mallory,[1] in a recent article on cirrhosis, as follows: "Apparently uncomplicated bile stasis does not result in any sclerotic process around the hepatic veins where the chief lesion is located and it certainly does not around the portal vessels." This may be true for man; yet, if it be true, the reaction or lack of reaction in the liver would seem to form the only exception to the general rule that obstruction of the duct of a secreting gland leads to a (numerical) atrophy of the gland with secondary sclerosis of the organ. The liver in the rabbit forms no such exception. Ligation of the common bile-duct in the rabbit, without demonstrable succeeding infection, results in a cirrhosis of the liver.

The experiments here reported, which bear upon this assertion, are a portion of a series undertaken for the study of the regenerative phenomena occurring in the liver subsequent to injury of the organ. It was hoped to gain some information as to the factors which determine the variations in regenerative activity seen in different types of cirrhosis, in order to ascertain, if possible, why in some cases regeneration of the parenchyma is so marked a feature, while in others proliferation is apparently confined to the duct epithelium.

Among the procedures employed to injure the liver was ligation

[1] *Bull. Johns Hopkins Hosp.*, 1911, xxii, 69.

of the duct, and it is with the changes which follow such ligation that this paper chiefly concerns itself.

In performing these experiments, the rabbits have been placed under ether anesthesia, which has usually been of less than thirty minutes' duration. Under as nearly aseptic conditions as possible, the peritoneal cavity has been opened by a high median incision, and the common bile-duct located and ligated a short distance from its duodenal end, precaution being taken to avoid any manipulation of the liver which might lead to its injury. Double rows of sutures have been used in closing the abdominal wound. The lesion result-ing has been studied at the end of twenty-four hour, forty-eight hour, one week, two week, and one month periods. All of the animals showed jaundice twenty-four hours after ligation, and in some cases it was very marked. The animals which were allowed to live beyond the first two days became progressively more and more emaciated, and at the end of two weeks they were almost devoid of subcutaneous or retroperitoneal fat. The animals seemed to have no inclination to eat for some time after the operation. Aside from jaundice and emaciation, there have been no post mortem changes which seem to need description in organs other than the liver, and, in consequence, general protocols of the autopsies are omitted.

Within twenty-four hours after the ligation of the bile-duct, well marked changes have taken place in the liver. In animals killed at that time, the gall-bladder and the bile-ducts are distinctly distended with clear, light green bile. The liver is definitely larger than normally and has a mottled appearance, opaque, light colored spots being set in the rather deeper brown general background. On sec-tion, there is a marked general cloudiness with a mottling due to the same opaque spots seen from the surface. These dots and areas of considerable size are scattered throughout the liver section. The lobulation of the liver is somewhat obscured.

The character of the change becomes apparent upon study of the microscopical sections. The liver cells generally are swollen and almost every cell has two nuclei, which is the result, as shown by study, of direct division of the nucleus. The most striking feature, however, is the presence of areas of necrotic cells, corresponding

to the opaque areas seen in the gross specimen. These areas apparently begin at the edge of the portal spaces and extend toward the central vein. In some places connective tissue of the portal space as well as cells of the lobules is involved; in other places it is not. The areas vary much in size and shape. They may include only six or eight cells in any one section, or they may involve one third of the lobule, and extend to the central vein. They tend, as a rule, to be oval in outline, but vary much from this usual shape. The necrosis appears to be of a peculiar type,—quite different from the hyaline necrosis so often seen in man. The cells stain very lightly with eosin, so that only their outlines are distinct. The nuclei either fail to stain or else appear as shrunken eosin-staining dots, while the protoplasm is represented by fine, faintly staining granules. At the edges of these areas, one finds in some places a sharp transition to more nearly normal cells, and in others, transition stages in which the first change appears to be a shrinkage of the nucleus leading to complete pyknosis. The cytoplasm in these transition stages also begins to stain less intensely. A Marchi stain for fat shows that in the larger areas of necrosis the liver cells toward the center are free from fat droplets, but that toward the periphery of the areas the capillaries and the capillary endothelium contain many fine and fairly coarse droplets.

There is in these areas of necrosis some invasion by leucocytes, which are found not only between the cells but also invading them. In some areas definite hemorrhages are found. In addition to these larger areas of necrosis, scattered necrotic cells may be found throughout the lobule. The portal spaces show dilatation of the bile-ducts and a spreading apart of the connective tissue fibrils by a fluid exudate. There is no leucocytic reaction about the ducts, nor are leucocytes found within them.

During the succeeding twenty-four hours there is an intensification of this picture, and some further important changes. The areas of necrosis are more numerous, their shape is more irregular, and they are larger. In some places the major part of a lobule is included in one area of necrosis. The hemorrhages into the areas are more numerous and more extensive. The individual cells of the larger necrotic areas show at this time no trace of a nucleus.

The shadowy particle seen at the end of twenty-four hours has disappeared. The outlines of the cells themselves have in many places become indistinct and even invisible, while the protoplasm of most of the cells is extremely tenuous, and appears to be undergoing rapid autolysis. In the central and, in greater numbers, in the mid-zone of the lobule, are scattered isolated cells showing either marked fatty degeneration or necrosis.

At this forty-eight hour period there is no sign of regenerative change in the parenchyma, as no mitoses could be found in the liver cells after very careful and protracted search. Conditions in the portal spaces, however, are quite different. Mitotic figures in the bile-duct epithelium are numerous, and bile-duct sprouts begin to appear. There is also even at this time an increase in cells of a fibroblastic character in the connective tissue of the spaces.

At the end of a week, there are gross signs in the liver of atrophy and scarring. The surface of the organ begins to have a slightly granular appearance, with slight depressions separating parenchymatous nodules. The liver is lighter in color and still shows a mottling with opaque, light yellowish dots. These are also evident on section. Upon microscopic study of the liver in this stage, areas of apparently recent necrosis are still found corresponding to those described for the earlier stages. Some of these areas are quite large and may even involve whole lobules and parts of adjacent lobules. Other areas of necrosis show complete disintegration of the cellular elements. These, as a rule, appear smaller than the recently necrosed areas and show in many places invasion by fibroblasts which have sprung from the tissue of the portal spaces. In some places, also, columns or cords of liver cells penetrate a short distance into the necrotic areas. Isolated necrotic liver cells are present throughout the lobule, as in earlier stages.

The portal spaces in this stage are much altered. The bile-ducts show a greater degree of proliferation, which is still in active progress, as is shown by the numerous mitotic figures in the duct cells. The bile-ducts radiate toward the edge of the liver lobules and show connection with and apparently differentiation into cells typical of the liver parenchyma. The connective tissue of the portal spaces has increased much in amount, and, as a result of its tendency to

invade the lobule in the areas of necrosis, and also at times to grow between apparently normal liver cells, the portal space begins to have a stellate appearance. In addition to the dilatation of the bile-ducts there is also a dilatation of the blood vessels of the portal space.

The picture at two weeks' time from the tying of the duct is practically an intensification of that after one week, as far as the growth of bile-ducts and of connective tissue is concerned. In one respect, however, it differs markedly. The larger areas of necrosis which formed so prominent a feature in the earlier stages are almost entirely lacking. Individual necrotic cells, however, are still found in abundance.

The last stage in the process studied was four weeks after the tying of the duct. At this period the bile-ducts are extremely dilated and tortuous. The common duct averages about seven millimeters in diameter. The gall-bladder is also much increased in size and tensely filled with clear green bile. It measures, roughly, five centimeters in length and between two and three centimeters in its widest part. The liver itself does not appear to vary much from the normal in size. It is light yellow in color and very granular in appearance. It is firm and cuts with difficulty,—the typical liver of an established cirrhosis. On microscopical examination, the most striking features of the liver are the smallness of the lobules and the increase in the portal spaces. With the removal of the necrotic cells, there has been a great reduction in the size of the lobule generally, as well as a distortion of the lobules in many places. The cells of the center of the lobule and, in fact, of all of the lobule save of the outer two or three rows, appear degenerated. Some are necrotic, others show fat vacuoles, while others merely stain more lightly and are loosely granular in appearance. The outer rows of cells have a much more normal appearance and seem to be continuous with the newly formed bile-ducts of the portal space. These latter are very numerous; indeed, the portal space appears to contain a thick network of the ducts. Where a distinct lumen is shown in the duct, bile pigment is often seen. The ducts branch toward the edges of the lobule and terminate in many places in cords of typical liver cells.

The connective tissue of the portal spaces is evidently still increasing. It still has a young appearance, and in no place has it the dense, scar-like appearance of old cirrhosis cases in man. The small parenchyma lobules are completely surrounded by connective tissue and are in many places invaded by fine strands which separate isolated liver cells and cords of cells.

In considering these experiments and the stages of the cirrhosis here described, in an attempt to determine the pathogenesis of the lesion, there seem to be few points of real difficulty, though some must still perhaps be left as debatable. The primary injury is very definite. It consists of scattered areas of necrosis of liver parenchyma, involving also capillaries and often some of the connective tissue of the portal spaces, beginning at the edge of the portal spaces and extending toward the central vein. There appears to be no doubt but that this primary lesion is due to the escape of the dammed back bile from the weakest point of the biliary conducting-system, *i. e.*, the juncture between the bile capillaries and the ducts proper. Whether this bile acts directly upon the liver cells or primarily upon the capillaries, producing a secondary anemic necrosis of the liver cells, is still questionable, though there are several features which indicate that both actions take place. The experiments of Dr. Bunting and Dr. Brown in this laboratory show that rabbit bile once elaborated is very toxic to rabbit tissues, even to the liver when applied to it externally. It will also cause necrosis of vessel walls and thus thrombosis. The lack of any relation of some of the irregular necrotic areas to blood supply and blood flow, and the occurrence of small areas consisting of a very few cells and even of individual cells, might indicate direct action. On the other hand, in some cases definite thrombi were found in the portal vessels. Furthermore, the occurrence of dilatation of portal vessels in later stages would indicate interference with the circulation within the lobules, as it occurs before there is sufficient connective tissue growth in the spaces to impede flow. In this primary injury one has all the elements that Mallory considers necessary for the production of a cirrhosis,—injury to parenchyma, to connective tissue, and to blood vessels; there might be added tension of the tissues due to dilatation of the ducts.

In the reaction to this injury, the bile-duct proliferation is the most marked early feature, though the connective tissue growth begins at the same time. The immediate stimulus to the bile-duct epithelium would appear to be the increased tension due to the damming back of bile. That there is a very active proliferation is shown by the large number of mitotic figures found early and even after two weeks' time. In fact, bile-duct proliferation appears to be the chief regenerative feature on the part of the epithelial elements. I am not inclined to favor the term " pseudo-bile-ducts " for the resultant growths. As MacCallum[2] has shown, in acute yellow atrophy in man, the proliferating bile-ducts show a distal differentiation into typical liver cells. In man, this attempt at regeneration appears to be abortive. In these experiments in the rabbit, however, it appears less so. The distal ends of the ducts become differentiated into cords of liver cells in some places; in others, they seem to unite directly with the existent cells of the lobule. Their lumina contain bile. In the latest stage studied, regeneration from the bile-ducts seemed to be responsible for the more normal liver cells forming the periphery at the lobule. Regeneration on the part of the parenchyma, however, appears in these experiments to have been very slight. No mitoses were found in parenchyma cells at any stage, though they were carefully searched for. That there was some regeneration the sections show definitely, in the invasion of necrotic areas by columns of liver cells, but it falls far short of that seen after a single toxic injury, and the gradual shrinkage in the size of the liver lobules would seem to indicate that the disturbance in function of the cell and the damming back of the toxic bile were handicaps too great for the parenchymatous cells to overcome. The proliferation of connective tissue elements was prompt and progressive throughout the period studied, mitoses being still present in the one month stage. Finally, in no case was any microscopical evidence of infection of the bile-ducts found.

In conclusion, I desire to thank Dr. Bunting for the interest he has taken and for the assistance he has given throughout the course of this study.

[2] *Johns Hopkins Hosp. Rep.,* 1902, x, 375.