

EXPERIMENTAL BACILLARY CIRRHOSIS OF THE LIVER.¹

By PROFESSOR LUDVIG HEKTOEN.

From the Pathological Laboratory, Rush Medical College, Chicago.

WITHOUT entering upon the broad questions of the etiology and genesis of cirrhosis of the human liver, I wish to refer briefly to recent demonstrations made in this laboratory of cirrhotic processes in the livers of animals, directly induced by two different bacilli. These observations seem to me to be of interest, from their suggestiveness of the possibility of a somewhat similar course of events in some instances of human cirrhosis. Similar more or less acute infections in man may induce a diffuse growth of new connective tissue in the liver. While the bacteria may be destroyed, and the immediate effects of their presence pass away, the newly formed tissue in the liver remains, and, as it contracts, a vicious circle is established—the pressure of the resulting fibrous tissue causes necrobiosis of the hepatic cells, which in turn leads to renewed, more or less slow but progressive connective tissue proliferation. With time, and perchance aided by other superinduced factors, extensive contraction of the liver may result.

One of the observations referred to was conducted by Dr. G. H. Weaver, and has been published by him.² The bacillus belongs in the colon group, and was isolated from a guinea-pig dying spontaneously. The bacillus unfortunately lost its virulence early, but enough experiments were made to establish clearly the relation between the necrotic and the proliferative changes in the liver. In the animals that succumbed early, necrotic and degenerative changes only were present; in those living longer, proliferation of connective tissue was associated with necrosis; and in other animals cirrhosis entirely replaced the degenerative changes. These interesting effects were

¹ An abstract of this paper was presented to the Pathology Section at the Annual Meeting of the British Medical Association by the Hon. Sec., as a contribution to the discussion on "Cirrhosis of the Liver," 2nd August 1900.

² "Cirrhosis of the Liver in the Guinea-pig produced by a Bacillus and its Products," *Trans. Chicago Path. Soc.*, 1900, vol. iii. pp. 228-335; "Contributions to the Science of Medicine by the Pupils of William H. Welch," 1900, pp. 297-305.

observed only in guinea-pigs, showing well the influence of species, and were caused by inoculation of living as well as of devitalised cultures.

The second observation concerns a bacillus that may be placed in the pseudo-diphtheria group. It was isolated from the lesions of a case of blastomycetic dermatitis of the back of the hand. Inoculations in various ways with this bacillus and its products have been found to produce with a fair degree of constancy more or less necrosis and diffuse cirrhosis of the liver in guinea-pigs and other animals. Unfortunately this bacillus also lost its virulence before many of the experiments planned with it could be completed. It would have been of great interest to have had the chance to follow the subsequent course of the cirrhosis set up in some of the animals.

THE BACILLUS.

MORPHOLOGY.—The bacilli are about four or five times as long as they are thick; a few are longer than others, especially in the older cultures; in twenty-four hour glycerin-agar cultures the bacilli are quite uniform in size. Stained with Löffler's methylene-blue, the majority of the bacilli present a typical palisade form, owing to the presence of stained zones alternating with unstained; some show rows of stained granules in their interior, sometimes only one at each pole; others are irregular in shape, diffusely stained and clumpy, especially in older cultures; a few are club-shaped, and twice the ordinary length; some are slightly curved, but most are straight. On the smears the organism has a tendency to arrange itself in parallel groups of twos, fours, or more. The bacillus stains well with Gram's method. It does not show any granules in its interior when stained by Neisser's method. The bacillus is non-motile.

BIOLOGY.—*Glycerin-agar plates.*—After twenty-four hours the colonies appear as small points, the largest about pinheads in size. Under the microscope the surface colonies are circular, light yellowish or brownish in the centre, and solidly granular, the periphery being lighter, the margins irregular. After forty-eight hours the outer half of many colonies is of a creamy colour, granular, numerous clear spaces separating the granular masses.

The deep colonies are smaller, oval or circular, brownish, and not so granular as the superficial ones.

Gelatin plates.—After seventy-two to ninety-six hours, brownish, granular, circular, and sharply-outlined colonies appeared.

Glycerin-agar.—A moderate, whitish-yellow growth, with rounded, slightly raised wavy margins.

Agar-agar.—A spreading, moist, slightly raised growth, which is not quite so extensive as that on glycerin agar.

Glucose-agar.—A slight growth along the stab, with a moderate mass on the surface; no formation of gas.

Gelatin.—A filmy, whitish growth along the stab; no liquefaction.

Potato.—A slightly raised, dirty whitish growth, along the needle track; after forty-eight to seventy-two hours the growth becomes more raised and brownish in colour.

Alkaline bouillon.—Bouillon becomes diffusely clouded, a film forms on the surface, and there settles at the bottom a heavy, greyish-white, or yellowish viscid sediment, which slowly increases at the same time as the medium becomes a little less turbid.

Litmus-milk.—There is no coagulation. On the fourth day the colour turns light reddish in some tubes.

Production of acid.—In neutral and alkaline bouillon no acid is produced, as determined by the phenolphthalein method.

Fermentation.—No gas is produced in glucose, lactose, or saccharose bouillon, prepared according to Theobald Smith's formulæ.

Indol.—A slight amount of indol is produced in Theobald Smith's bouillon.

Temperature.—An exposure to a temperature of 59° C. for three minutes kills the organism.

Anærobiosis.—The organism grows fairly well in Buchner's jars.

ANIMAL EXPERIMENTS.

Guinea-pigs.—Subcutaneous injection of 1 to 1.5 c.c. of bouillon culture generally produces in from two to four days a marked, hard infiltration, followed by the development of more or less extensive, punched-out ulcers, which gradually heal. The animals become thin, and die in from three to five weeks. The essential changes are necrosis and cirrhosis of the liver. The bacillus is generally to be recovered from the internal organs and from the cutaneous ulceration. This course of events is illustrated by the following experiment:—

On 7th October a guinea-pig received subcutaneously over the abdomen 1½ c.c. of a bouillon culture of the bacillus. On the following days it seemed sick, and gradually a huge infiltration occurred along the track of the needle, with desquamation of the skin. On 12th October two large ulcers had formed with clear-cut margins, the floor being covered with a greyish exudate, from which inoculations on glycerin-agar gave rise to pure growths of the bacillus. The ulcers gradually healed, and the animal became apparently well. It died, however, 26th October, greatly emaciated. Pure cultures of the bacillus were obtained from the lungs, liver, and kidneys. The liver presented a finely granular surface, with here and there very small yellowish foci.

Microscopic examination.—There is a well-marked portal cirrhosis; quite broad bands of rather cellular fibrous tissue surround the vessels in Glisson's capsule. There seems to be but very slight formation of new bile ducts. There is no distinct focal necrosis in the sections. The intralobular capillaries contain fairly numerous polymorphonuclear leucocytes, and the hepatic veins are filled with blood. The epithelium of the convoluted tubules of the kidney is rather granular and swollen.

Occasionally local reaction failed to take place, the animals at times recovering; at other times foci of necrosis would develop in the liver. In a few cases local infiltration and ulceration took place, but the other changes were not found.

In an animal that was killed thirteen days after the inoculation of 4 c.c. of an old culture, the following changes were found in the liver, which showed many yellow spots:—

The large yellow foci consist of huge leucocytic accumulations, enclosing small masses of, and single, liver cells, which are more or less hyaline, stain pinkish with eosin, and show a faintly stained nucleus. Surrounding these foci is a recent zone of well-developed granulation tissue. There are small foci of necrosis and connective tissue proliferation throughout the liver, both at the periphery and in the central parts of the lobules. There are occasional extensive portal connective tissue proliferations, with the formation of new bile ducts, but no regular, widespread cirrhosis.

Similar general results may follow intraperitoneal injection, though not so constantly.

On 27th September a small piece of blastomycetic skin was inserted into the abdominal cavity of a guinea-pig. The cultures from the skin contained the bacillus in large numbers. The animal died, 25th October, greatly emaciated. There were no local changes at the site of the operation. The organs contained the bacillus in pure culture. The liver presented an uneven surface, with rather large, irregular patches of whitish-yellow colour, and of softer consistence than the rest of the organ, which was firmer than normal.

Microscopic examination.—There is a moderately extensive recent perilobular connective tissue proliferation, associated with a moderate degree of hyperplasia of the biliary passages. There are quite a number of round foci of necrosis in the interior of the lobules; scattered among the nuclear fragments in these foci are some cells with vesicular nuclei and oval cell bodies. The intralobular capillaries in some places contain groups of cells with deeply stained nuclei. The irregular yellow patches noted on the surface are structureless, necrotic districts, at the margins of which there is a moderate cell infiltration.

The kidney, spleen, and lungs show no changes. Twenty-four hours after intraperitoneal injection of 1 c.c. of a fresh bouillon culture there is a slight increase of fluid, but the peritoneum is smooth. The spleen is large and its capsule tense. The liver shows a few irregular, pinhead sized, yellowish-white dots. Smears of the peritoneal fluid show a number of leucocytes only; those from the spleen show a number of disintegrated, large round cells, filled with bacillary masses of various sizes, the bacilli being very closely packed together. The cultures from all organs remain sterile. Histologically all organs appear nearly normal. The epithelium of the kidney is somewhat swollen, and the spleen is cellular. The liver cells appear granular and swollen, but the nuclei are intact; the vessels are much congested.

In specimens of livers, from animals killed on the third and sixth days after intraperitoneal injections, there are scattered throughout the organ numerous minute foci of necrosis, with nuclear detritus and cells with vesicular nuclei. These areas may occur in the interior or at the margin of the lobule. With Gram's stain small bacillary masses are seen in some of these areas. There is some periportal connective tissue proliferation in some places. In some of the sections, branches of the portal vein and hepatic artery appear markedly contracted, leaving a rather homogeneous zone between the vessels and the liver substance. The junction of this zone with the liver is occupied by cells with

greatly distorted nuclei, and a few distorted nuclei are scattered about throughout the more homogeneous area. The intralobular capillaries contain many cells with long drawn out, deeply stained nuclei, and there are a few small areas of cells with fragmented nuclei in the lobules.

In these cases the peritoneum was found smooth, and both smears and cultures from the various organs failed to show any bacteria. The injection into the peritoneal cavity of 1 c.c. of bouillon culture, heated to 60° C. for fifteen minutes, in some cases produced a few small areas of necrosis of the liver after two to four days.

Similar results were obtained with cultures filtered through porcelain. Death rarely resulted from toxine injections alone, in doses of 1 or 2 c.c. In some of these cases the appearances described in the following experiment were noted :—

A guinea-pig, weighing 312 grms., received into the peritoneum 1 c.c. of a forty-eight hour bouillon culture, heated to 60° C. Fifteen days later it weighed 305 grms.; it then received 2 c.c. of the heated culture. Two days later it weighed 298 grms., and 2 c.c. were again injected. Three days later it died, weighing 290 grms.; cultures sterile.

Histological examination.—Zenker's fluid, hæmatoxylin, and eosin.—The liver shows great swelling of the parenchymatous cells, the outlines of which are indistinct, the cell bodies faintly granular, the nuclei pale and vesicular. There are many leucocytes in the capillaries; small accumulations of cells are found around some of the central veins, and not infrequently there is a broad zone of cellular fibrous tissue around the vessels in Glisson's capsule. At the border of such zones the liver cells present a peculiar radial arrangement, the bodies being drawn out quite long, and sometimes they appear to send out protoplasmic processes, the nuclei being smaller, more deeply stained and compact than elsewhere, the cell bodies staining more intensely red with eosin. The lungs show heaps of cells, with round, deeply stained nuclei, about the medium-sized vessels. The spleen and the kidneys appear quite normal. Bacteria could not be demonstrated in the liver or lungs by Gram's method.

Repeated injections of heated or filtered cultures, in doses of 2 c.c. on alternate days, were made, followed after a varying time by the injection under the skin of 1 c.c. of twenty-four hour bouillon culture; local reaction and emaciation were not produced, and in no case were the necrosis and cirrhosis of the liver as marked as described in the early experiments.

Diphtheria antitoxine does not exercise any protection, as far as can be judged from the following experiment :—

On 14th October a medium-sized guinea-pig received subcutaneously 1 c.c. of a twenty-four hour bouillon culture of the pseudo-diphtheria bacillus, and 150 units of diphtheria antitoxine. It died 6th November. Pure cultures of pseudo-diphtheria bacilli were recovered from the liver and the spleen, the lungs containing the *Staphylococcus aureus*. The liver had an irregularly depressed surface and a yellowish softened focus in one of the left lobes. The lungs were much congested and the upper left lobe contained pneumonic areas. The kidneys contained much blood.

Histological examination.—The liver shows an advanced perilobular cirrhosis. Broad bands of fibrous tissue surround the lobules, and send narrow prolongations among the rows of cells in their interior. The fibrous tissue is quite mature, and its stroma stains well by van Gieson's method. In addi-

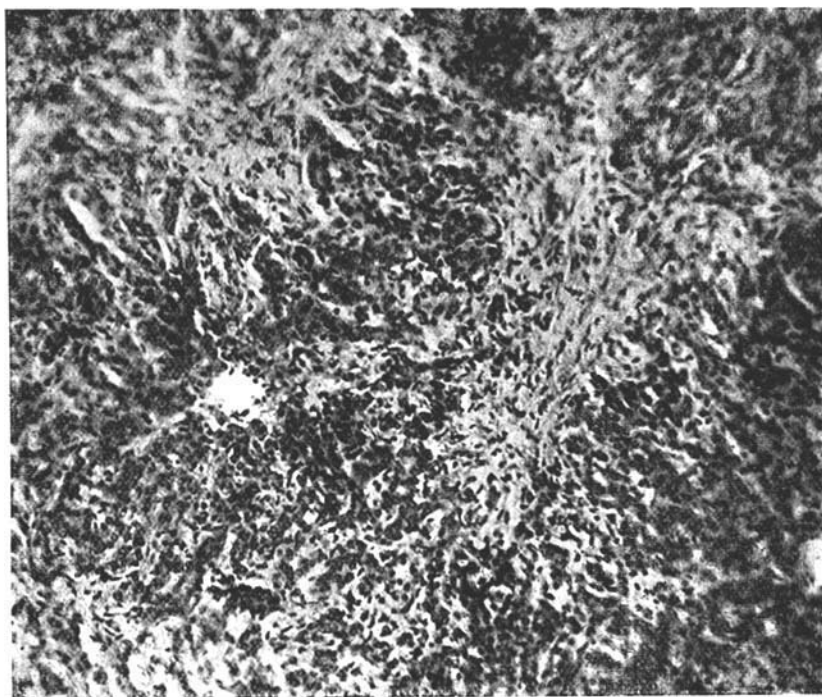


FIG. 1.

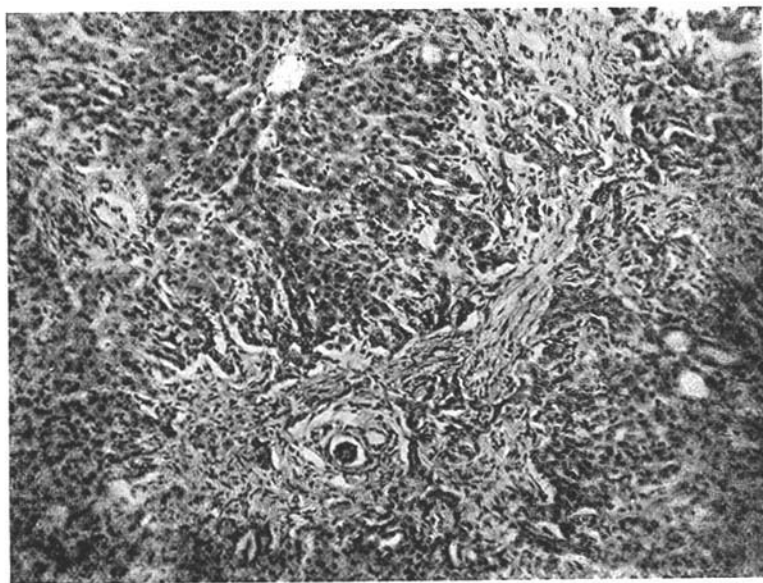


FIG. 2.

tion to older and younger connective tissue cells this new tissue also contains irregular masses of epithelial cells, which resemble liver cells; groups of three to ten or fifteen cells, lie in spaces in the stroma; sometimes the cells tend to form a central lumen. Occasionally the new fibrous tissue encloses small homogeneous and structureless necrotic districts, and the yellow focus referred to above is of similar appearance.

The pneumonic area in the left upper lobe shows the alveoli filled with a cellular exudate, in which are many nuclear fragments and colonies of cocci. The kidneys show some granular degeneration of the epithelium of the convoluted tubules. The spleen is congested and somewhat cellular; there are no foci of necrosis in the spleen.

RABBITS.—The intravenous and subcutaneous injection of 1 c.c. of the culture did not make the animals sick nor produce any changes in the liver. Inoculation into the anterior chamber of the eye of a full-grown medium-sized rabbit resulted in great emaciation and death after twenty-four days, with cirrhotic and other changes in the liver.

At first the inoculated eye showed a well-marked hypopyon, which gradually subsided until a small white area remained in the anterior chamber, the cornea appearing quite normal.

Post-mortem.—There are pneumonic areas in the right lung; the liver is shrunken, irregular on the surface, and contains firm yellow spots. The pseudodiphtheria bacillus was isolated in pure culture from the lungs and the liver, the other organs being sterile.

Histological examination.—Zenker's fluid, hæmatoxylin, and eosin. The numerous firm yellow areas in the liver, which appear as lighter red districts in the sections, correspond to the greatly thickened and cellular periportal tissue. There is a well-marked and universal connective tissue proliferation about the vessels in Glisson's capsule, with the production of more or less extensive patches of firmer fibrous tissue. There is also a desquamation of the epithelium of the large bile ducts, as well as of many biliary channels. The newly formed fibrous tissue encloses small groups of cells which look like liver cells, and also cells with distorted and broken-up nuclei and loose nuclear spheres. The liver cells proper are cloudy and swollen, and among them lie isolated cells with nuclei that have undergone rhexis. The lungs show an extensive broncho-pneumonia with more or less nuclear fragmentation, sero-fibrinous exudate, and marked congestion. Efforts to demonstrate the bacilli in the sections from the liver and the lungs by Gram's stain resulted negatively.

DOG.—Subcutaneous injection of 2 c.c. of the culture gave rise to a huge swelling, which gradually subsided. The animal became very fleshy during its confinement, and was killed with chloroform six weeks after the injection. The liver was of about normal size, finely uneven, with occasional single greyish dots or groups of dots. There was slight grating on cutting. Microscopic sections showed numerous small intralobular islands of recent connective tissue cells, as well as of small cells with deeply stained irregular nuclei. There were also branching bands of recently formed connective tissue along the vessels.

Inoculations of a grey mouse and of a white rat with 0.2 c.c. and 0.3 c.c. respectively of a bouillon culture made both animals sick for a time, but when killed on the twenty-first day the livers were found normal.