MORPHOLOGICAL AND FUNCTIONAL PHASES IN THE DEVELOPMENT OF EXPERIMENTAL CIRRHOSIS OF THE LIVER INDUCED BY CARBON TETRACHLORIDE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 54, No. 9,
pp. 110-114, September, 1962
Original article submitted November 1, 1961

Of the various methods of inducing cirrhosis of the liver experimentally, the simplest and most effective is to give repeated doses of specific liver poisons. With carbon tetrachloride it is very easy within a comparatively short space of time to induce cirrhosis of the liver, accompanied by ascites and other typical manifestations of portal hypertension [1-9].

We here report the development of morphological and functional changes in the liver, portal vein, spleen, and other organs and systems in relation to the amount of CCl_4 given, and to the time elapsing after its administration.

EXPERIMENTAL METHOD

The work was done on 36 dogs weighing 7-26 kg. Through a stomach tube, 2-5 ml* of CCl_4 were given twice weekly. A control group consisted of six dogs. At various times after the start of the treatment, and between $2\frac{1}{2}$ and 9 months after the end of the course, repeated laparotomies were performed in order to take a liver biopsy specimen, and to measure the pressure in the portal vein. All material for histological study was fixed in 10% formalin. Sections were cut either on a freezing microtome, or else cut after hardening and embedding in celloidin. The preparations were stained in hematoxylin-eosin or resorcin-fuchsin, using Weigert's method. Some of them were stained in Sudan III, or by Foot's silver method.

Before and after treatment, systematic observations were made on the change in weight, condition of the blood, and the biochemical changes in the serum.

EXPERIMENTAL RESULTS

It was found that the dogs varied greatly in sensitivity to CCl_4. Some seven of the dogs readily tolerated 20-25 doses, and outwardly did not differ from healthy dogs. Of the others, after 2-5 doses 20 became weak, ill, ate badly, or even took no food for several days and rapidly lost weight. Finally, 9 died with acute dystrophic changes of the liver (subacute or acute yellow atrophy of the liver), or of other vitally important parenchymatous organs.

Histological investigation of the preparations of biopsy samples of liver frequently revealed a number of morphological changes. The variation was due firstly to individual sensitivity and a difference in the degree of the response, and also to layer upon layer of destructive changes in the organ, related to the repetitive application of the poison. Nevertheless, from the whole of the histological material collected we could not fail to notice a number of systematic pathological changes in the liver, related to the amount of the injected poison and to the time which had elapsed after the experiment.

Thus, 7-14 days after 2-4 doses of CCl_4 had been given, biopsy liver specimens examined microscopically showed almost complete disappearance of the trabeculae and loops, and at the same time showed a development of profound dystrophic changes of the hepatic cells. In addition to an advancing necrosis and breakdown of the hepatic cells, quite frequently the blood vessels also were necrosed in regions showing numerous petechial hemorrhages, or sometimes extensive bleeding into the parenchyma (Fig. 1, A, B).

By the 21st day, after 6-7 injections of CCl_4, in addition to continued destructive changes in the liver (partial necrosis of liver cells and vacuolization of the remainder), further events had occurred. The regions of small-cell infiltration had extended, especially where there was widespread necrosis of the hepatic cells and regeneration of the parenchyma.

*Dogs weighing 7-10 kg, 11-15 kg, 16-20 kg, and more than 20 kg received 2, 3, 4, and 5 ml respectively.

By the 30th day after the start of the experiment (after 8-9 doses of CCl_4), the liver was noticeably enlarged, firm to the touch, and slightly granular. Microscopically, a diffuse vacuolization of the hepatic cells was observed. Frequently parts of the interlobular stroma were thickened by small-cell infiltration and outgrowth of young granulation tissue. There was also a marked hyperemia, while the intralobular veins and deep capillaries were engorged, forming local convolutions (Fig. 1, C, D).

After 37 days from the start of the treatment (after 12 doses), in a biopsy liver specimen, besides the cytoplasm being strongly vacuolated, the interlobular stroma was thickened through the accumulation of lymphoid plasma cells, and the outgrowth of granulation tissue. This change extended all the way from the periphery to the central veins (Fig. 1, E).

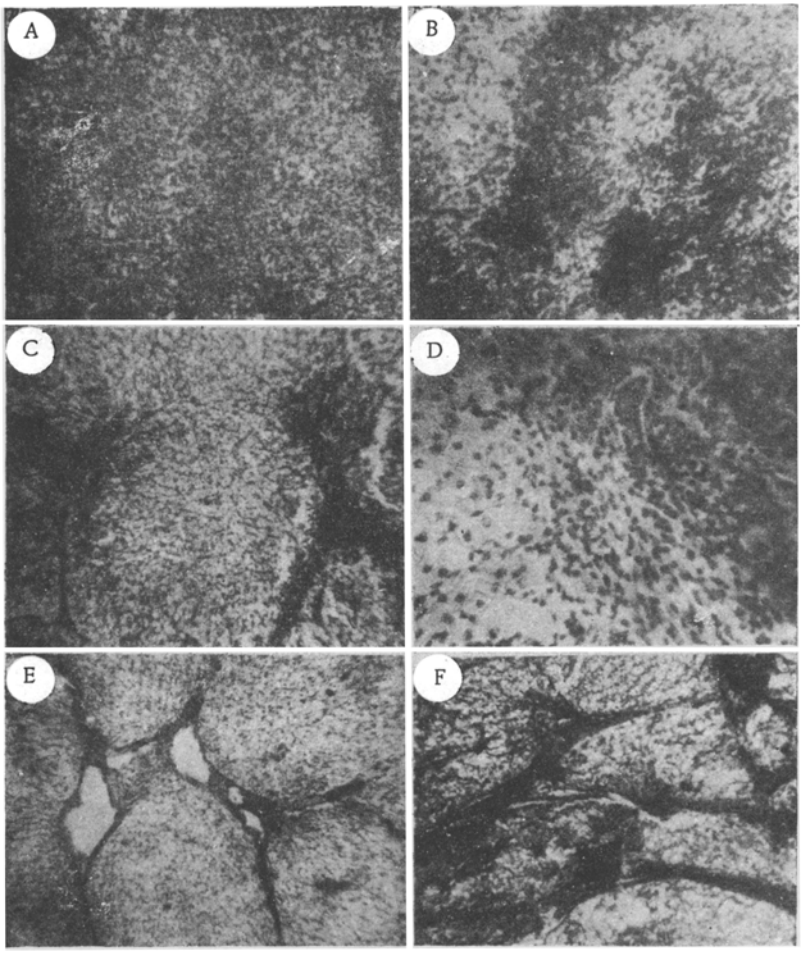

Fig. 1. Pathological changes in the liver at various times after CCl_4 had been given. A) Marked destruction of liver tissue, 7th day; B) disintegration of hepatic cells, and extensive hemorrhages, acute toxic hepatitis; C and D) partial necrosis and vacuolization of the remaining hepatic cells. Areas of small-cell infiltration in the interlobular stroma, 30th day; E) thickening of the interlobular stroma through the accumulation of lymphoid and plasma cells, at the outgrowth of maturing granulation tissue, 37th day; F) changes of the capsular type of cirrhosis of the liver, 50th day; A – C, E, F) eyepiece 7×, objective 8×; D) eyepiece 7×, objective 40×.

By the 50th day, after 16 doses of CCl_4, a considerable outgrowth of stroma along Glisson's capsule could be seen under the microscope, where the delicate fibrous strands anastamozed with each other. Many of the central veins were thickened and constricted. Around them could be seen numerous outgrowths of immature connective tissue, rich in capillaries, and of varied cellular composition (Fig. 1, F).

Later, after all the CCl_4 had been given, signs of continued dystrophic changes in the hepatic cells could be observed; they were not so intense, but were maintained for a long time. However, after a certain time, the number of cells with vacuolized protoplasm or signs of necrosis was gradually reduced, as they were replaced by regenerating hepatic cells, normal in shape and in staining reactions. At the same time there was a progressive increase of the sclerotic changes typical of capsular cirrhosis of the liver. Thus, 64 days after the CCl_4 treatment (on the 11th day after all the CCl_4 had been given), when the fourth laparotomy was performed the liver was found to have an uneven surface with a strikingly nodular surface. A biopsy specimen showed connective tissue growing out around the lobules. It was more mature, and contained more fibrous cords, most of which were penetrated by clumps of hemosiderin.

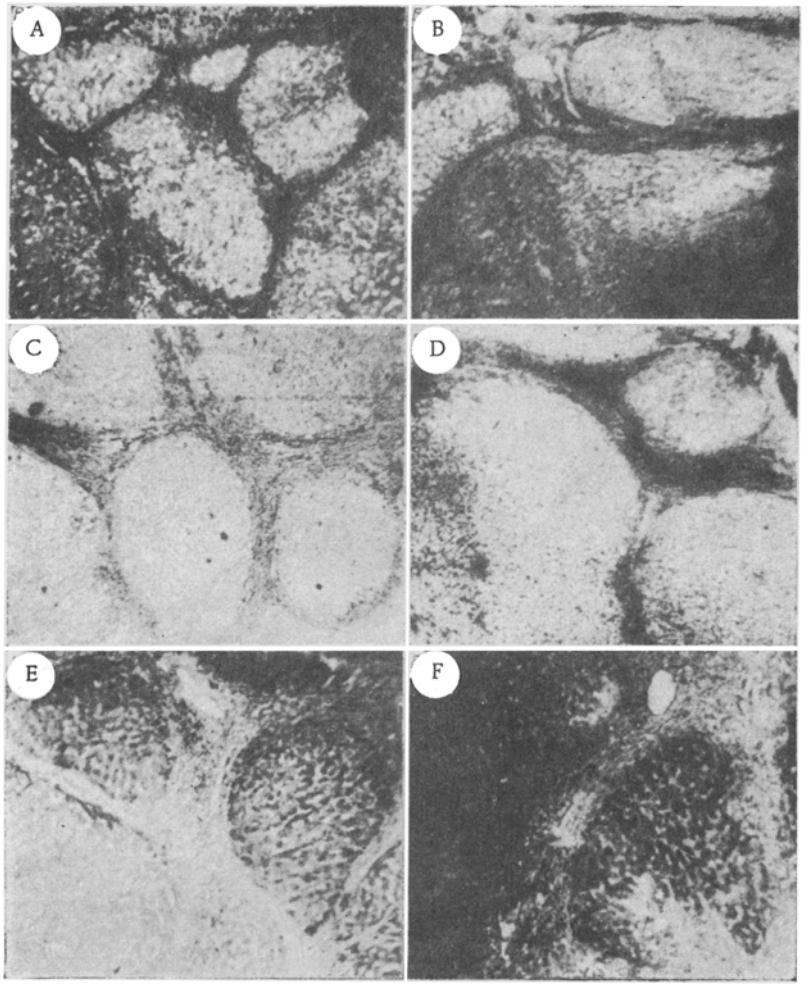

Fig. 2. As in Fig. 1, but later on in the experiment. A) Considerable outgrowth of Glisson's capsule between the lobules and around the vessels where it forms narrow cords of fibrous tissue, 80th day; B) extensive outgrowth of more mature fibrous connective tissue, 100th day; C) 3rd month; D) 5th month; E and F) 9 months after the start of the experiment; further increase of sclerotic changes in the liver, typical of capsular cirrhosis. Ocular 7×, objective 8 ×.

On the 80th day after the start of the experiment (7 days after all the poison had been administered) histological examination of portions of the liver showed an extensive outgrowth of Glisson's capsule between the lobules around the vessels, where it took the form of narrow cords; there was also an outgrowth of connective tissue within the lobes, and formation of islets of hepatic parenchyma of abnormal structure, and of false lobules (Fig. 2, A).

One hundred days after the start of the experiment, the connective tissue which had grown out between the lobules appeared as maturing granulation tissue and more mature fibrous tissue. As in previous observations, in parts the hepatic cells were in a condition of atrophy, showing vacuoles, dystrophic changes and necrosis, and in parts they were regenerating (Fig. 2, B).

Still later after the start of the experiment, and 6 to 7 months after all the CCl_4 had been given, microscopic examination of biopsy liver specimens showed a picture closely resembling human capsular cirrhosis (Fig. 2, C–F). There was a massive outgrowth of Glisson's capsule between the lobules and around the vessels, as well as a proliferation of fibrous connective tissue within the lobules, and formation of atypical islets of hepatic parenchyma and false lobules. The central veins within the lobules lay eccentrically, sometimes two in one lobule, while in some cases they were absent entirely. To the naked eye the liver appeared small, firm to the touch, and having a coarsely granular or nodular surface.

Numerous measurements of the portal pressure made during liver biopses, and at the end of the experiment showed the extreme sensitivity of the response of the portal vein to all changes occurring in the liver (see Table).

The portal pressure began to rise as early as a few days after the development of toxic hepatitis; subsequently, as the morphological changes progressed, and particularly when cirrhosis developed, the pressure reached very high values, leading to the syndrome of portal hypertension.

Changes in Portal Pressure (in mm water) during the Development of Experimental Toxic Hepatitis and Cirrhosis of the Liver

No. of dog	Time after first administration of CCl_4 (in days)														
	7	11	14	21	25	30	37	40	50	64	80	120	150	210	270
133	147	—	159	—	—	186	—	—	—	—	215	—	—	280	—
121	139	—	151	—	—	170	177	—	—	200	—	228	—	—	—
134	—	135	—	142	—	—	—	205	—	—	—	—	—	—	310
141	—	154	—	158	—	—	190	—	—	—	—	290	—	—	—
136	—	—	127	—	153	—	—	—	192	—	—	—	245	—	—

Ascites developed in 9 of the 27 dogs with cirrhosis. It was readily apparent from the outward appearance, and the amount of fluid formed was 1.5-3 liters. In many of the animals the tortuous dilated veins of the belly and thorax stood out, and could be felt.

In the dogs which had undergone prolonged CCl_4 poisoning the liver also underwent many pathological changes (fibroadenia) but they were not always very marked. Often the spleen was noticeably enlarged, and was firm to the touch. There was also a dilatation or even a tortuosity of the splenic veins, and a dilatation of the lumen of the portal vein and of many of its branches. A study of biopsy spleen specimens 4-9 months after the start of the poisoning by CCl_4 showed a noticeable thickening of the capsule and of its trabeculae. The Malpighian bodies had atrophied, and were reduced in number. The lumens of the sinuses were filled with desquamating endothelium. The pulp was sclerosed to various extents. Around the sclerosed vessels, fibrous nodules had developed. The animals lost weight particularly rapidly by the end of the first month after the start of the experiment. The weight then continued to fall, and finally became stable by the 3-5th month after the start. During this time the loss in weight ranged from 1000 to 3700 g, representing 7-18% of the original weight.

As a rule, even 2-3 months after the start of the experiment, in most of the dogs signs of anemia developed. The number of erythrocytes fell to 3 or 4 million per ml. Hemoglobin dropped to 70-41%. At the same time, at the start, as the toxic hepatitis developed there was a marked increase in the number of leucocytes to 21,000 or more,

after which the number gradually fell to 3000-5000 until in certain cases a full leucopenia developed. Accompanying these changes there was also a reduction in the number of thrombocytes to 200,000 to 130,000. As the cirrhosis developed, there was a marked increase in the rate of clotting of the blood.

We were able in this way to follow not only the stages in the development of experimental cirrhosis of the liver induced by prolonged poisoning with CCl_4, but also to obtain the after-effects of an enlarged spleen, portal hypertension, ascites, etc.

SUMMARY

A toxic hepatitis leading to cirrhosis of the liver was induced in 36 dogs by prolonged administration of carbon tetrachloride. Biopsy specimens were taken periodically during the whole period of a long-term experiment; portal pressure, splenic and blood changes, and weight were observed. Observations were made not only on the developmental stages of the cirrhosis, but also on such complications as hypersplenism, portal hypertension, ascites, etc.

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