EXPERIMENTAL DATA ON THE GENESIS OF CIRRHOSIS OF THE LIVER DUE TO DIETS DEFICIENT IN CHOLINE AND METHIONINE

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As a result of clinical and experimental investigations, considerable data have been collected relating to the importance of an alimentary factor in the genesis of disease of the liver. The study of experimental cirrhosis due to choline and methionine deficiency gives grounds for thinking that in human pathology conditions are possible which could lead to the onset of cirrhosis of the liver with a similar (or closely related) genesis [2].

However, in the problem of experimental cirrhosis due to dietary deficiency of sources of active methyl groups (choline, methionine), there is a number of problems on which opinions of investigators are conflicting. Some workers consider that cirrhosis is preceded by extensive necrosis of liver tissue* arising with or without fatty infiltration of the liver [7,9]. Meanwhile, experimental results show that the development of this type of necrosis in the liver is brought about by deficiency of S-containing amino acids but not of methyl groups, and that the consequences of this deficiency may to a certain extent be prevented by administration of vitamin E [8]. The question whether the presence of necrosis is really an essential condition for the development of cirrhosis remains unanswered.

Another question awaiting examination concerns the role of fatty infiltration. Some workers consider that the presence of prolonged fatty infiltration fulfils the conditions of development of cirrhosis and also that severe fatty infiltration is transformed into cirrhosis through a stage of "extracellular lipohepatosis", characterized by the appearance of masses of so-called fatty cysts. However, facts exist which demonstrate that the necessary conditions of development of cirrhosis are not fulfilled by fatty infiltration alone. For this reason investigation is necessary into the question whether additional factors besides the formation of fatty cysts do not precede experimental alimentary cirrhosis.

Finally, an explanation is also required of the nature of the resemblance between this particular form of experimental alimentary cirrhosis which we are examining and the cirrhosis observed in man. Some writers [7,3] consider that the pattern of experimental cirrhosis is close to that of cirrhosis in man, while others [11] think that the pathological conditions are only slightly comparable.

* This refers to extensive lesions of parts of a lobe or of whole lobes with necrosis of the epithelial and connective tissue parts of the organ. Dystrophic changes in the liver cells, undergoing fatty infiltration, in some cases terminating in their death, do not enter into the meaning of the expression "necrosis of liver" in this instance.

In order to answer these questions we performed the experiments described below.

EXPERIMENTAL METHODS

The experiments were performed on 70 male white rats weighing initially 40 to 90 g. We used the diet which was introduced by Daft [6] for production of cirrhosis. Some additions were made to the composition of the ration.

The dietary mixture which we used consisted of the following portions: casein (4%), lard (8%), corn starch (84.5%), salt mixture (3%) [10], cystine (0.5%). The animals received the following vitamins daily: thiamin (100γ), riboflavin (50γ), pyridoxin (20γ), calcium pantothenate (20γ), nicotinic acid (1 mg), vitamin A (20 international units), vitamin D (8 international units). Once a week each rat was given 3 mg of vitamin E. Inclusion of vitamin E in the diet was with the aim of preventing the development of necrosis of the liver as mentioned above.

We also had the intention of reproducing alimentary cirrhosis under conditions pathogenetically relatively close to those in which a similar type of cirrhosis may arise in man. Investigations showed that deficiency of protein in the diet involves the development of endogenous choline insufficiency [4]; for this reason we included in the diet a small quantity of protein. However, in view of the fact that in small quantity casein is insufficient on account of its lack of S-containing amino acids [12], we included cystine in the diet. Tryptophan deficiency could arise; we compensated for this to some extent by the addition of nicotinic acid. The increased amount of thiamin in the diet was on account of its high carbohydrate content.

In order to study the successive phases of the changes in the liver, we periodically performed biopsy. For this purpose, under ether anesthesia we made a small incision in the epigastric region and delivered the left lobe of the liver. We excised a small piece measuring 0.5 cm^2; the omentum was applied to the wound in the liver to control hemorrhage. The peritoneum and muscular wall were closed with continuous sutures and the skin with interrupted sutures. In not one out of 30 cases of biopsy, including some repeated operations, did the animal die. In addition, some organs (liver, lungs, kidneys) of animals killed at various stages of the experiment were examined under the microscope.

Histological preparations (fixation in 10% formalin, imbedding in paraffin and gelatin) were stained with Sudan III by Goldman's method, hematoxylineosin, picrofuchsin mixture, carbolfuchsin (ceroid) and were also impregnated by the Tibor-Pan-Snesarev method.

The maximal length of time spent by the animals in the experiment was 16 months.

EXPERIMENTAL RESULTS

In the first 3 months was noticed the development of severe fatty infiltration of the liver. The data relating to this period are from specimens obtained from 28 animals; in 18 of them biopsy was carried out.

The first biopsy was performed on the 3rd day of the experiment. Macroscopically the liver was clay colored; microscopically small droplets of fat were seen in the peripheral portions of the lobules. At the end of the first month, these portions were in a state of severe fatty infiltration, while the protoplasm of the liver cells close to the central veins contained only small droplets of fat. During the following 2 months the severe fatty infiltration spread to the central zones of the lobules and acquired a diffuse character. At this time numerous fat emboli (Fig. 1) began to appear in the capillaries of the lungs; in individual cases fat was discovered in the lumen of the small intrahepatic bile ducts. The deposition of fat in the vascular and biliary systems was connected with the appearance in the liver of so-called fatty cysts, the formation of which and the possibility of rupture into the vascular and biliary systems were described by W. Hartroft [14]. In addition to fatty infiltration were also seen necrosing liver cells with disappearance of the membrane and dissolution of the nucleus. At this period also was formed the pigment ceroid, staining a crimson color with carbolfuchsin. Pigment granules were seen in the connective tissue of Glisson's capsule and along the course of the hepatic veins.

The state of the liver in the next period of the experiments – between the 4th and 12th months – was also studied by us in 28 animals. Without reducing its size, the liver became superficially much more granular.

Microsopically, beginning on the 5th-6th month, the quantity of visible fat in the majority of cases diminished sharply. This process was accompanied by the appearance of numerous diffusely scattered foci of regeneration (Fig. 1. b), and in the protoplasm of the newly formed liver cells no fat could be demonstrated. Signs of marked cirrhosis were present in the 10th-12th months.

In the next period of observation – between the 12th and 16th months of the experiment – changes in the liver were studied in 14 rats; five of them had a preliminary biopsy. In the majority of the animals of this group, the pattern of cirrhosis of the liver had developed, consisting of a combination of dystrophic and regenerative changes, diffuse sclerosis and complete reorganization of the normal structure of the liver.

However, in individual animals (rats Nos. 35, 56 and 61) the pattern of severe fatty infiltration without sign of cirrhosis continued to be maintained. In histological preparations of the liver in these cases were found small and larger (up to 42 μ in gelatin) fatty cysts.

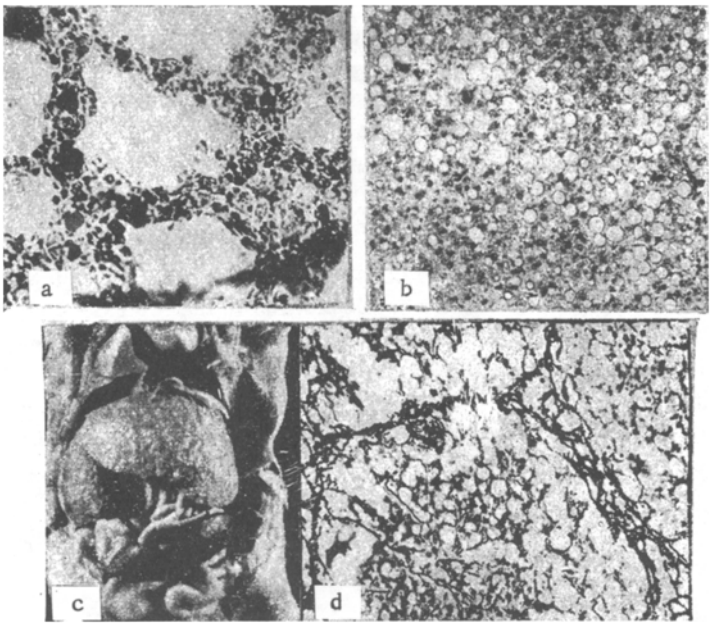

Fig. 1. Tissue changes in experimental cirrhosis in rats. a) Fat emboli in the interalveolar capillaries of the lungs on the 78th day of the experiment. Rat No. 25. Stained with Sudan III and hematoxylin. Magnification 80; b) small foci of regeneration against a background of fatty infiltration of liver cells still present on the 171st day of the experiment. Rat No. 41. Stained with hematoxylineosin. Magnification 80; c) cirrhosis of the liver (naked eye specimen); d) structure of liver tissue 16 months after the experiment. Impregnation by Tibor-Pan-Snesarev method. Magnification 100.

The total number of these cysts was not large and intracellular fat was more prevalent. In the zone corresponding to the most severe fatty infiltration, i. e., the periportal zone, thickening and coarsening of the argyrophilic fibers was noticed (Fig. 2). By comparison with cases where development of cirrhosis had occurred, in the liver of these animals attention was drawn to the weakly-expressed process of regeneration of the parenchyma. In these cases regeneration did not lead to the production of a large number of nodules of newly-forming parenchyma; it consisted of the disorderly new formation of small and large cells, sometimes of multinucleated liver cells, diffusely scattered in the parenchyma and, as a rule, also affected by fatty infiltration. In spite of the severe degree of the latter and the absence of a trabeculo-lobular structure pattern,

no reorganization of the vascular tree could be seen in the liver; there was no coarse, diffuse sclerosis. All these circumstances do not allow the changes observed in these animals on the 12th-16th months to be regarded as cirrhosis.

Necrosis was not observed, neither in early stages nor in the period of maximal development of fatty infiltration, even if this was maintained for 12 to 16 months.

Thus the experiments showed that the animals developed severe fatty infiltration of the liver, and against this background the pattern of cirrhosis took shape. This cirrhosis appeared to be a combination of dystrophy, regeneration, diffuse sclerosis and reorganization, i, e. signs by which the true cirrhosis of the liver in man is characterized [1].

Earlier we pointed out the opinion of some workers, that the development of cirrhosis is preceded by necrosis of the liver. In our experiments we avoided necrosis, giving the animals vitamin E, and in every case cirrhosis developed without preceding widespread necrosis. As a result we may consider that such necrosis is not a necessary condition of development of this type of cirrhosis of the liver.

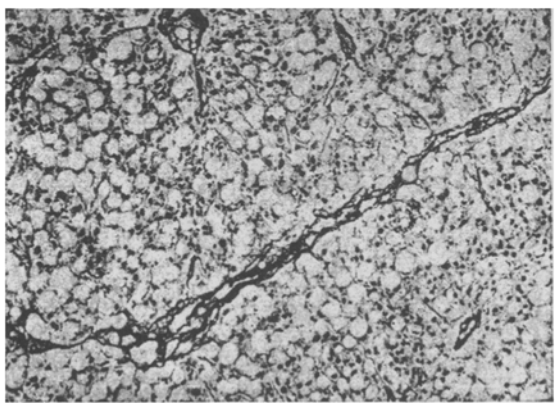

Fig. 2. Coarse argyrophilic fibers in the periportal zones of the liver of rats with experimental cirrhosis of the liver.
Duration of the experiment 14½ months. Impregnation by the Tibor-Pan-Snesarev method. Magnification 80.

Scrutiny of the successive microscopic patterns of change in the liver enables one further conclusion to be made. Some workers, studying the phases preceding the development of cirrhosis, have fixed their attention on the fatty infiltration of the liver, and have connected cirrhosis genetically with later transformations of the fatty cysts. From this point of view, in our experiments we must note cases in which, in spite of the long duration of the experiment, with severe fatty infiltration and the presence of fatty cysts, cirrhosis did not develop (in 3 out of 14 rats in experiments lasting from 12-16 months). It is very important that in these cases we did not observe any marked degree of regeneration. From these findings, we consider that in addition to the formation of fatty cysts, in the production of cirrhosis as studied in our experiments an essential role is played by regenerative processes.

The results of our experiments draw attention to one further fact. In the experimental rats we did not find cirrhosis before 10-12 months after the beginning of the experiments, while according to Canadian and some American workers cirrhosis develops in the animals in the 4th month of the experiments. This may probably be explained by the fact that the choline and methionine requirements of rats of different strains may vary [5].

SUMMARY

Cirrhosis of the liver, which occurs as a result of prolonged deficiency of choline and methionine in food, developed in those experimental animals in which intensive process of regeneration of the parenchyma took place together with considerable fatty infiltration. Necrosis of the liver is not a required condition in development of the cirrhosis of the liver which was studied,

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