Fate of Implanted Collagen in the Liver of White Rats. (20013)

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Observations of several investigators suggest that the quantity of fibrous tissue in the liver of animals with experimental cirrhosis may diminish spontaneously(1,2) or may be influenced by favorable nutritional conditions (3-5). A study of the reversibility of fibrosing processes is hampered by the variability in the development and intensity of experimental cirrhosis in the individual animals or any experimental series.

The present study was undertaken to find out whether collagen can be absorbed in the liver. For this purpose a standardized procedure was employed whereby a) a known quantity of collagen was available for absorption by the liver; b) the process could be accurately dated; and c) extrahepatic factors could be eliminated, such as general symptoms of poisoning, disturbances of food intake and utilization or alterations of the hepatic vasculature.

Material and methods. In 22 male rats between 180-240 g a single collagen thread attached to a non-traumatic needle was implanted transversely into the large left lobe of the liver. Twelve rats received flexor or extensor tendons which were removed from the hind feet of a freshly killed young adult rat and immediately implanted. Ten rats received plain surgical gut, U.S.P. (sheep collagen), size 3/0 which was supplied heat sterilized and sealed in ethyl alcohol containing mercuric iodide of 1:2000. Before use the threads were washed with sterile physiologic saline. Only minimal hemorrhage resulted from the operative procedure and all animals recovered immediately following closure of the abdomen. The animals of both series were sacrificed in pairs at 1, 2, 3, 4, and 8 weeks. In addition, rats with implanted tendon were sacrificed 12 weeks after the operation. Two blocks of tissue from the left lobe of each liver were examined histologically. Sections were stained with hematoxylin and eosin and Laidlaw's silver method counterstained with van Gieson.

Results. 1. *Implantation of homologous tendon.* The tendon remained unchanged in size and apparently viable during the first 2 months of observation. In one animal sacrificed at the end of this period extensive calcification appeared in the tendon (Fig. 1). In one rat examined at the end of 3 months the tendon appeared still viable but slightly narrower in diameter than in all other animals. With regard to the absence of regressive changes or of invading granulation tissue we would consider this difference in size to be due to smaller caliber of the tendon at the time of implantation as compared with the rest of the tendons. A narrow capsule of collagen tissue separated the tendons from the hepatic parenchyma in the livers of all rats. The adjacent parenchyma showed no evidence of compression or cytological alterations.

2. *Implantation of surgical gut.* The implanted surgical gut excited a reaction in the liver characteristic of a foreign body. At first it was surrounded by a wall of neutrophilic leukocytes, cellular granulation tissue and foreign body giant cells. A wide zone of fibroblasts had formed at the periphery between which were numerous concentrically arranged argyrophile fibers, most dense adjacent to the hepatic parenchyma (Fig. 2). Within 2 weeks the gut was fragmented and invaded by granulation tissue and foreign body giant cells. In the periphery a capsule of collagen fibers had developed which showed silver-impregnated fibrils in lesser amount than before.

By the third week the thread of gut was in greater part replaced by a cellular granulation tissue including a small number of collagen fibers and surrounded by scarce concentric

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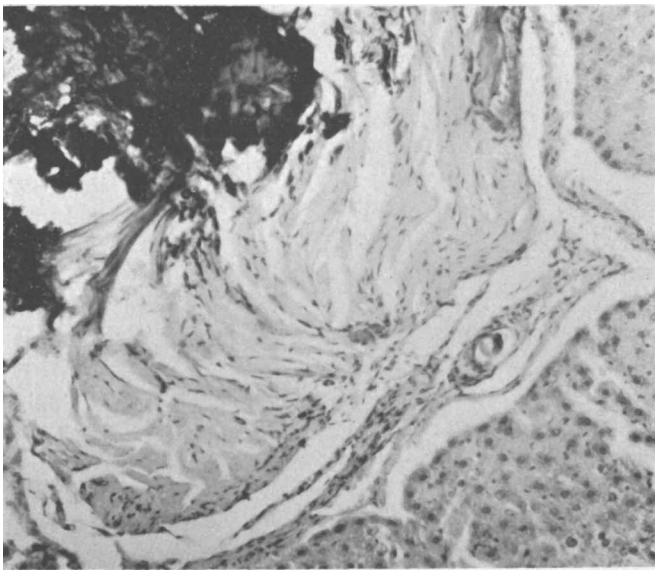

FIG. 1. Homologous tendon in liver, 2 months following implantation. The tendon is in part calcified and surrounded by a narrow rim of newly formed connective tissue. Hematoxylin and eosin. × 150.

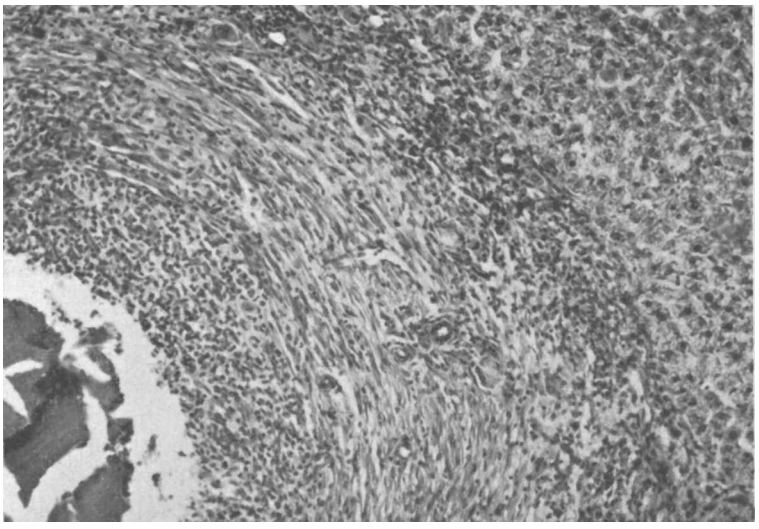

FIG. 2. Surgical gut in liver one week following implantation. A wide capsule has formed around the gut consisting of abundant fibroblasts and argyrophile fibers. Hematoxylin and eosin. × 150.

bundles of argyrophile fibrils (Fig. 3).

During the remainder of the observation period the granulation tissue was gradually converted into a small nodule of loose fibrous tissue containing a few hemosiderin-filled macrophages (Fig. 4).

Discussion. Homologous collagen in the form of tendon is apparently an inert material and excites little or no reaction from the hepatic tissue. It remains unchanged in size and shape after implantation into the liver. This part of the present experiment, therefore, does not indicate whether fibrous tissue formed in the liver may regress spontaneously or not.

Surgical gut (sheep collagen), on the other hand, is rapidly replaced by invading granulation tissue and foreign body giant cells. The significant feature of this process is that

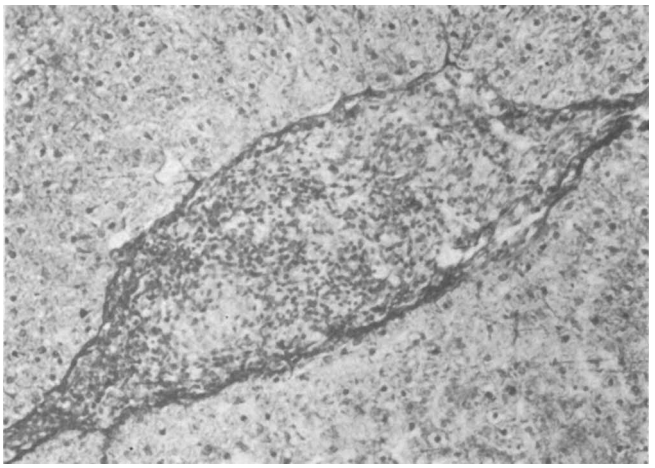

FIG. 3. Three weeks following implantation the gut is almost entirely absorbed. Only a few reticulum fibers are still present. Laidlaw-van Gieson's stain. × 150.

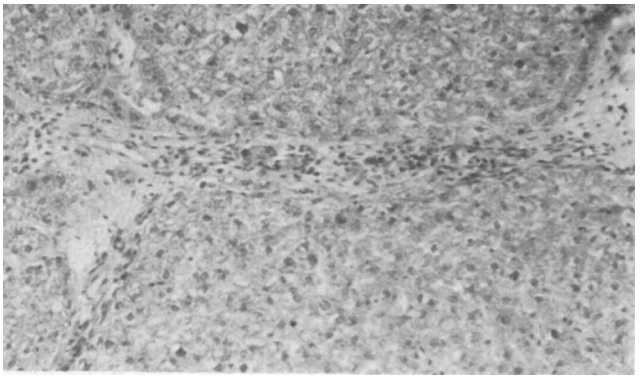

FIG. 4. Two months following the operation a small, loose scar had remained at the site where surgical gut was implanted. Hematoxylin and eosin. × 150.

initially, up to one week following the operation, there is proliferation of fibrous tissue around the foreign "gut" which later almost entirely disappears. The wide zone of reticulum and collagen fibers which surround the gut are probably due to actual proliferation and not to a condensation of preformed connective tissue.

The regression of the newly formed fibrous tissue demonstrated in the present experiments supports the observations on the possibility of spontaneous "reversal" of experimental cirrhosis in the liver of rats which has been demonstrated by quantitative chemical determinations by Morrione(6).

Summary. 1. Homologous tendon and plain surgical gut were implanted into the livers of young adult rats for the purpose of investigating the capacity of the organ of absorption and new formation of collagen fibers at measured intervals. 2. Homologous tendon caused no remarkable reaction in the liver and remained viable and in undiminished quantity for an observation period of 3 months. 3. Surgical gut was absorbed during about 3 weeks. At the end of the first weeks there appeared abundant newly formed collagen fibers around the implanted gut. These were observed in decreasing quantity during the following 7 weeks. 4. It appears that the normal liver has the capacity to absorb newly formed collagen fibers as well as non-viable collagen (surgical gut), but not homologous tendon collagen.

1. Cameron, G. R., Karunaratne, W. A. E., *J.*

Path. and Bact., 1936, v42, 1.

2. Steinberg, B., Martin, R. A., *Arch. Path.*, 1946, v41, 1.

3. Seller, E. A., Lucas, C. L., Best, C. H., *Brit. Med. J.*, 1948, v1, 1061.

4. Gyorgi, P., Goldblatt, H., *J. Exp. Med.*, 1949, v90, 73.

5. Jaffe, E. R., Wissler, R. W., and Benditt, E. P., *Am. J. Path.*, 1950, v26, 951.

6. Morrione, Th. G., *Am. J. Path.*, 1949, v25, 273.

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Competitive Inhibitive Action of Normal Fatty Acids on Mammalian D-Amino Acid Oxidase. (20014)

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During an investigation of the effect of various carboxylic compounds on the metabolism of mammalian tissues it was found that some of the simple normal fatty acids are competitive inhibitors of the mammalian D-amino acid oxidase. This action of the simple fatty acids does not seem to have been reported although Klein and Kamin(1) noticed an inhibition of D-amino acid oxidase by sodium benzoate. Later Bartlett(2) showed that the inhibition was competitive and that of many derivatives tested *m*-methyl benzoate was most effective. Zeller and Maritz(3) found that benzoate also inhibits ophio-L-amino acid oxidase and that other aromatic carboxylic and sulphonic acids had similar effects. The short chain normal fatty acids are shown to be more specific in their action; they inhibit the mammalian D-amino acid oxidase but have no action on ophio-L-amino acid oxidase.

Materials and methods. The source of the mammalian enzyme was a rat kidney homogenate. The animal was decapitated, exsanguinated, the kidneys removed and chilled on cracked ice. They were defatted, cut into small pieces and homogenized in 7.0 ml ice cold saline for 2 minutes. The homogenate was filtered through two layers of cheesecloth and 1.0 ml used per vessel. The substrate in all experiments reported was DL-methionine. The results were confirmed in most cases with DL-alanine as substrate, and it was shown that L-alanine was not oxidised by the rat kidney homogenate. The activity of the amino acid oxidase was followed in the Warburg apparatus by measuring oxygen uptake at 37°C with KOH filter paper in the centre well. In general, constant rates of oxygen uptake were found for 20 minutes and the inhibitions reported are inhibitions of this initial constant rate of oxygen uptake. Dried cobra venom was obtained from Hynson, Westcott and Dunning Ltd. (Baltimore) and 3.0 mg was used per vessel as the source of the ophio-L-amino acid oxidase.

Results. All the normal fatty acids from acetic to octanoic were tested as inhibitors of

TABLE I. Oxidation of DL-methionine by Rat Kidney Homogenate in Presence of the Sodium Salts of the Short Chain Fatty Acids.

Additions*	μl oxygen taken up in 20 min.	% inhibition
Nil	23	0
Methionine	146	
Sodium acetate	18	0
" " + methionine	145	
" propionate	18	0
" " + methionine	141	
" butyrate	24	39
" " + methionine	100	
" pentanoate	18	69
" " + methionine	56	
" hexanoate	15	50
" " + methionine	76	
" heptanoate	16	57
" " + methionine	69	
" octanoate	14	7
" " + methionine	128	

* All vessels contained 1 ml rat kidney homogenate, 1 ml .10 *M* phosphate buffer pH 7.4 and water to make a final vol of 3 ml. All the sodium salts were present at a final concentration of .01 *M* and the methionine at .02 *M*.