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CHRONIC LIVER DISEASE INDUCED IN RATS BY REPEATED ANAPHYLACTIC SHOCK

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A previous report dealt with the acute phase of liver damage resulting from anaphylactic shock in hyperimmune rats.[1] The rats in that study were made hyperimmune with human serum albumin and when shocked by intravenous injections of antigen, large periportal areas of necrosis developed in the liver (Fig. 1). Antigen and gamma globulin were demonstrated in sinusoids in damaged areas of the liver by fluorescent antibody examination. Subsequent studies showed that complement was also present.[2] As a part of the earlier study a small group of animals was repeatedly shocked and scarring of the liver was demonstrated. The present investigation is an extension of this observation, based on the postulate that repeated injections of sublethal doses of the antigen in the hyperimmune animal would result in scarring of the liver and in chronic liver disease.

The present study does demonstrate that repeated anaphylactic shock results in liver scarring. It also demonstrates that antigen may persist in small vessel walls for weeks after the last antigen injection and this observation suggests a mechanism of damage other than simply postnecrotic scarring and collapse.

MATERIAL AND METHODS

Seventy adult Holtzman rats of either sex, weighing 250 to 300 gm, were made hyperimmune with 3 injections, 5 mg each, of human serum albumin (HSA). This was given subcutaneously in incomplete Freund's adjuvant at 2-week intervals. Two

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weeks after the last injection, the animals were bled and the serum used for antibody titer determinations. The rats were then given weekly gradually increasing doses of HSA by tail vein. The dosages were gradually increased from 1 mg to 5 mg over a 12-week period and then continued at 5 mg until the end of the experiment. When the animals died or were sacrificed, tissues were obtained for histologic studies, which included hematoxylin and eosin, Masson trichrome and reticulum stains; tissues were also quick-frozen for fluorescent antibody studies. The techniques employed were as previously described.[1,3]

For control purposes animals of similar age and weight were immunized as described above and then were bled weekly by cardiac puncture to deplete the blood volume by approximately one-third. A shock-like state was regularly produced by this technique. At the end of the experiment animals were sacrificed as in the immunized group.

Through the courtesy of Dr. A. I. Braude of the University of Pittsburgh School of Medicine, the effect of endotoxin shock on the rat liver was evaluated. Sprague-Dawley rats given doses of endotoxin (*E. Coli*, 0113, Bovine) ranging from 0.5 to 32 mg died over varying periods up to 24 hours. Hematoxylin and eosin stained sections showed no necrosis or other significant lesions and, therefore, repeated shocking by this technique was not carried out.

To test for the presence in the serum in chronically shocked animals of a factor or factors which bound to nuclei or to cytoplasm of bile duct epithelium,[4] frozen sections of damaged rat liver from one of the chronically shocked animals and of human livers showing cirrhosis with bile duct proliferation, were employed. The whole rat serum obtained at the end of the experiment was placed on frozen sections of the livers, the excess serum removed by washing and fluorescent anti-rat globulin was applied as a stain to the liver. The sections were then examined with fluorescent light.

Results

All of the animals developed levels of hemagglutinating antibody and also precipitating antibody as determined by agar-gel diffusion. The hemagglutinating levels ranged widely from 1 to 1,000 to 1 to 106,000, but there was no correlation between these titers and severity of the acute or subsequent lesions.

The animals when given intravenous injections of antigen reacted as previously described with signs of shock characterized by dyspnea, a crouched position and ruffled fur.[5] Among the 70 animals in the experiment, only 44 survived 20 or more weeks. The other 26 animals succumbed to anaphylactic shock at various times earlier in the study. The actual cause of death in many of the animals surviving over 20 weeks was glomerulonephritis with uremia, and pneumonia. No consistent lesions were demonstrable in livers of the animals prior to 20 weeks; therefore, only animals surviving beyond this time are considered in the description and compilation of results.

The livers in rats surviving 20 or more weeks of shocking were grossly normal or finely granular. The weights were normal or slightly increased. In all there were varying degrees of fibrosis microscopically.

The mildest change, best demonstrated with the trichrome stain, was

a slight increase in collagen in periportal areas. More severe damage was seen as collagen fibers extended from the periportal areas into the lobules (Fig. 2). This reached a stage when lobular architecture was disrupted by bands of scar connecting central veins to portal areas. Areas of the liver were thus separated into pseudolobules (Fig. 3). Twelve of the 44 animals showed this lobular distortion. In all of these, reticulum stains showed foci or areas where collapse of lobules was suggested (Fig. 4).

The livers in 3 of the 44 rats contained areas resembling nodular regeneration (Fig. 5). These were recognized by increased eosinophilia of the liver cell cytoplasm. The nodules were small, usually no more than 20 cells in width and lay in the severely scarred areas, although not clearly related to collagen bands. In most of the livers, however, one could occasionally find smaller clumps, several cells in width, which suggested an early phase of nodular regeneration.

Bile duct proliferation was never prominent. Occasionally there was a mild ductal increase in a scarred portal area. A few ducts appeared slightly dilated (Fig. 6) and hyperchromasia of the lining epithelium suggested proliferation. Mitoses were rarely found. An inflammatory reaction usually became obvious by the 16th week in portal areas, but this was never a conspicuous feature. Inflammatory cells were lymphocytes or plasma cells (Fig. 6). These were never shown to contain specific antibodies to human serum albumin by the fluorescent antibody technique.

Antigen could be regularly demonstrated by the fluorescent method in small vessel walls 1 week following the last injection of antigen. To test for the persistence of antigen for longer periods, antigen injection was withheld from 4 rats for 4 weeks and then the animals were sacrificed. In these 4 rats, when the livers were stained with fluorescent antihuman serum albumin, antigen could be demonstrated in the walls of small arteries and veins in portal areas (Fig. 7) just as was the case in the more recently injected animals.

When the sera of 4 of the chronically shocked animals that had the more severe liver scarring were tested for the presence of anti-cytoplasmic or anti-nuclear factors,[4] the tests yielded negative results. No binding, as evidenced by fluorescence, occurred in either the damaged rat liver or in the human cirrhotic livers with bile duct proliferation.

Control animals shocked by bleeding over a comparable period of time showed no grossly visible liver lesions. Microscopically these were also normal except for a barely perceptible increase in connective tissue in the periportal areas in several livers; neither extension of bands of scar into lobules nor lobular distortion were found.

Discussion

The present report describes a method for producing liver scarring in rats. It is not a suitable model for the study of hepatic cirrhosis even if one might interpret these changes as indications of cirrhosis, because the animals died with nephritis. The physiologic alterations resulting from the nephritis precluded effective evaluation of those related to impaired liver function. The hepatic damage demonstrated was thought to be attributable to antigen-antibody complexes localizing in the liver sinusoids.[1] It might be, however, that the actual cause was occlusion of sinusoids by the antigen-antibody aggregates. As a result of the necrosis which sometimes became extensive, there was collapse of portions of liver lobules with resultant scarring and lobular distortion. The appearance of severe scarring was consistent with this postulate.

Another mechanism other than simple necrosis and collapse was suggested by the discovery that antigen might persist in small vessels. Antigen becomes widely disseminated at the time of injection and may reach the vessel walls then, although masses of antigen do localize in sinusoids. It was previously shown by radioactive tracer studies that antigen remained in the liver for long periods of time after it had been injected.[6] It was suggested in the study cited that the antigen was in liver parenchymal cells. Our findings suggested, however, that localization was in vessel walls. It is suggested, furthermore, that the persistence of the antigen in vessel walls might constitute a mechanism of damage comparable to that seen in certain forms of experimental glomerulonephritis.[7] The fine periportal scarring might be the result of this type of damage.

Summary

Hyperimmune Holtzman rats were repeatedly subjected to anaphylactic shock. These animals developed scarring of the liver with lobular distortion. Antigen was found to persist in the walls of small vessels in portal areas for as long as 4 weeks after the last antigen injection. It is suggested that in addition to postnecrotic scarring that the persistent antigen might act as an injurious agent and provide an additional cause of chronic liver damage.

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[*Illustrations follow*]

Legends for Figures

Unless otherwise indicated, sections were stained with hematoxylin and eosin.

Fig. 1. Liver, hyperimmune rat 12 hours after initial shocking with intravenous antigen. The acute phase is characterized by necrosis in a periportal area, hemorrhage and an infiltrate of neutrophils. × 100.

Fig. 2. Liver, chronically shocked rat. A portal area on the left exhibits increased fibrous tissue content. A wide band of dense collagen extends from here into a lobule. In the lower portion of the field is another band of collagen. × 100.

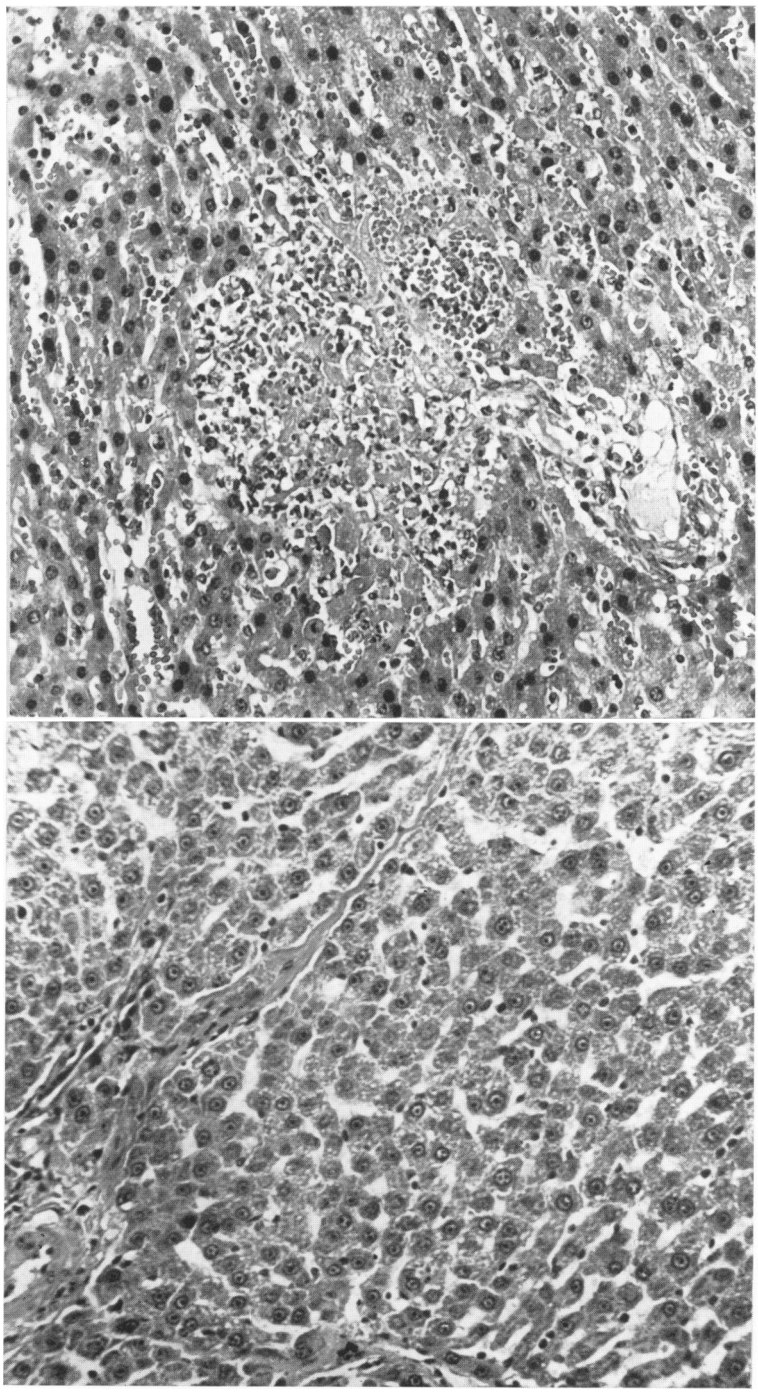

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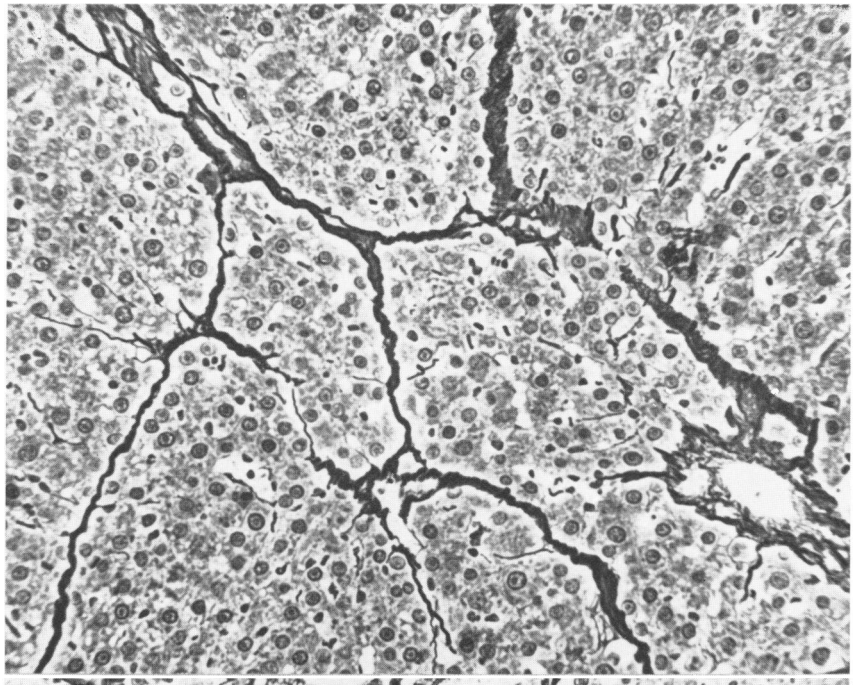
FIG. 3. Liver, chronically shocked rat. A portal area is on the right. Bands of connective tissue break up the liver into pseudolobules and fine bands radiate from the larger ones. Gomori's reticulum stain. × 100.

FIG. 4. A mass of collapsed reticulin occupies a large area in the center. Several small groups of liver cells may represent residual portions of lobules following collapse. Gomori's reticulum stain. × 100.



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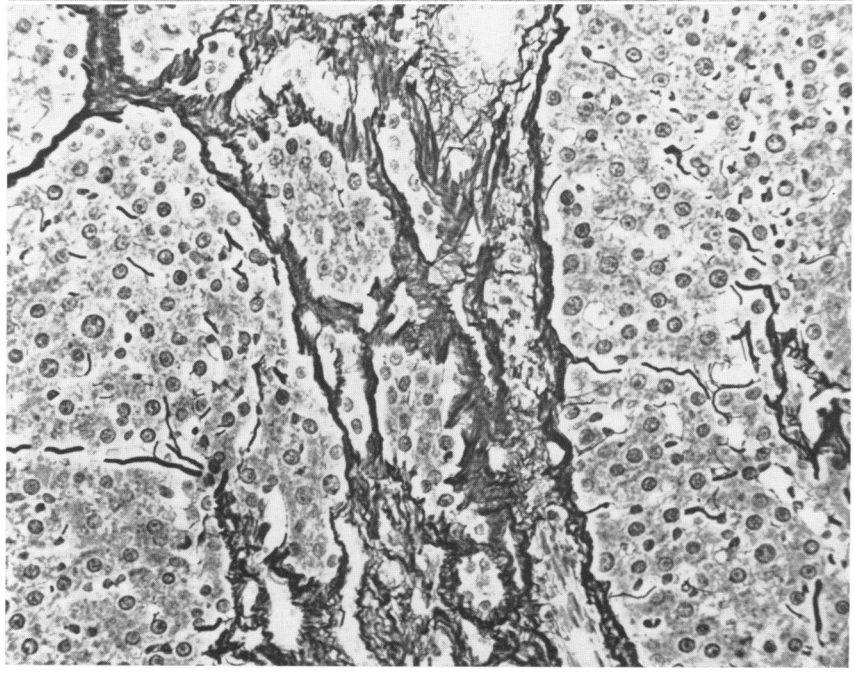

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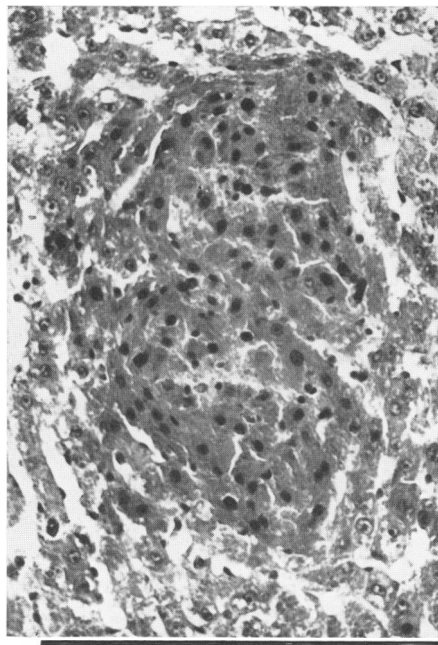

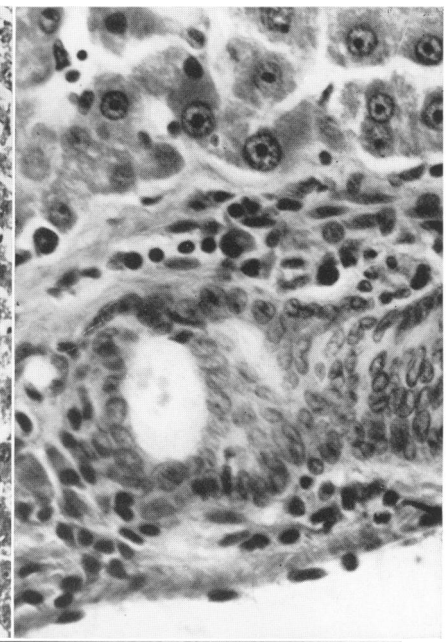

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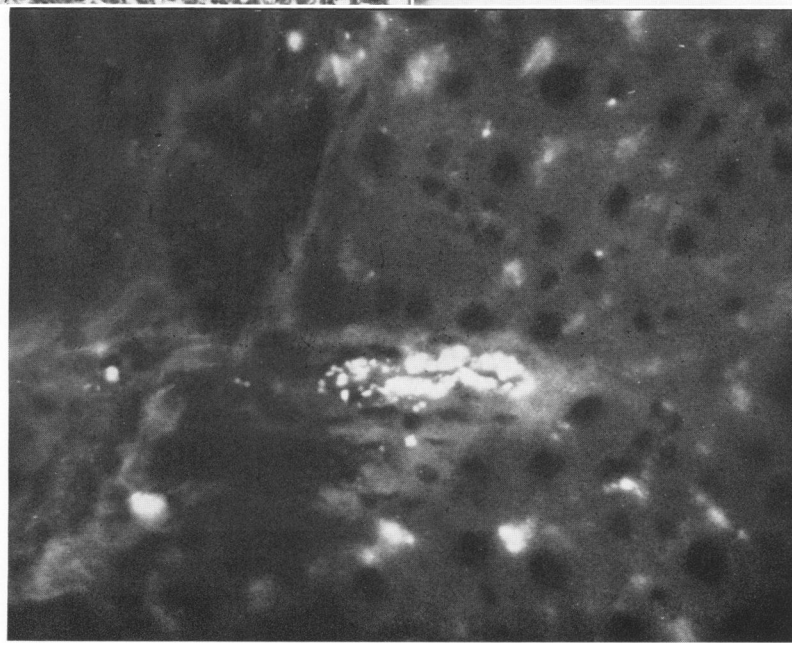

FIG. 5. An area of nodular regeneration consists of cells with dense, eosinophilic cytoplasm and dark nuclei. × 150.

FIG. 6. Inflammatory cells are conspicuous in a portal area. Connective tissue content is increased. The bile ducts are slightly dilated. × 250.

FIG. 7. A fluorescence micrograph of a portal area exhibits antigen persisting in the wall of a small vessel, probably an artery, 4 weeks after antigen injection. × 200.