The American Journal of
PATHOLOGY

AUGUST 1976 • VOLUME 84, NUMBER 2

Pathologic Changes in the Liver and Kidney Produced by Immunization With Intestinal Antigens

Bruce S. Rabin, MD, PhD, and Susan Rogers, MD

Rabbits were immunized with intestinal antigens derived from rabbits, guinea pigs, and germfree rats. Inflammatory changes throughout the portal tracts of the liver were found in 55% (16 of 29) of the immunized rabbits. Interstitial nephritis was present in 7 of 23 rabbits evaluated. These changes did not occur when nonintestinal antigens were used for immunization. Antigen shared by liver, kidney, duodenum, ileum, and colon were found in each of the species used for immunization. An immune response to the antigen shared by the various tissues may be a factor in the pathogenesis of disease in this experimental system. In man, chronic active hepatitis and interstitial nephritis are found in association with inflammatory bowel disease. A similar mechanism of pathogenesis may be a factor in these extraintestinal manifestations of inflammatory bowel disease in man. (Am J Pathol 84:201–210, 1976)

IMMUNE REACTIONS are thought to play an important role in the perpetuation of some types of liver disease. Chronic active hepatitis and primary biliary cirrhosis are associated with the presence of antibody to smooth muscle and mitochondria.[1,3] Antibody reactive with liver cell antigens may be found in these diseases[4,5] and cell-mediated immune responses to liver antigens have been reported.[1,2,4,6]

Experimental chronic active liver disease resembling that seen in humans has been produced by immunization of rabbits with lipoprotein

From the Division of Clinical Immunopathology, Department of Pathology, University of Pittsburgh School of Medicine, and the Department of Pathology, Presbyterian University Hospital, Pittsburgh, Pennsylvania.

Supported by Grant AM-18321-01 from the National Institute of Arthritis, Metabolic, and Digestive Diseases, US Public Health Service.

Accepted for publication April 6, 1976.

Address reprint requests to Dr. Bruce S. Rabin, Department of Pathology, Scaife Hall, University of Pittsburgh, Pittsburgh, PA 15261.

derived from human liver membranes.[7,8] In rats, a periductular fibrotic lesion in the liver has been reported to occur after immunization with autologous liver homogenate.[9] The lesion can be transferred to normal animals by viable spleen lymphocytes.[9]

In man, there is a significant association between chronic active hepatitis and inflammatory diseases of the intestine such as ulcerative colitis and Crohn's disease.[10,11] We have recently demonstrated that the intestine and liver share common antigens.[12,13] Immunization of rabbits with intestinal antigen derived from rabbits, guinea pigs, or germfree rats has produced histologic features of inflammatory liver disease. The immune response to the shared antigens may be a factor in the pathogenesis of the liver disease.

Materials and Methods

Animals

Female New Zealand white rabbits and female Hartley guinea pigs were maintained on Purina commercial feed. Female germfree rats were purchased from Microbiological Associates, Bethesda, Md.

Antigen

Animals were sacrificed, and the proximal 7 cm of guinea pig and germfree rat intestine and 15 cm of rabbit intestine distal to the pylorus were taken and designated as duodenum. Similar lengths of ileum were taken proximal to the cecum. Colon tissue was obtained from the descending colon.

The intestinal contents were removed by washing with phosphate-buffered saline (PBS), pH 7.4, and the tissue homogenized with PBS (2 ml/1 mg tissue) in a Sorvall Omnimixer. The suspension was stirred overnight at 4 C and centrifuged at 69,000*g* for 30 minutes. The supernatant was lyophilized and stored at 4 C. Lyophilized liver extract was prepared in an identical manner.

Antigen Immunization

Two milligrams of lyophilized antigen was dissolved in 1 ml PBS and emulsified with an equal volume of complete Freund's adjuvant. One milliliter of this mixture was injected subcutaneously into the intrascapular region of rabbits. The animals were immunized every 3 weeks for 6 months and terminally bled 3 weeks after the last injection. The sera were stored frozen at −20 C.

Histology

Liver, kidney, and intestine were fixed in buffered formalin and stained with hematoxylin and eosin, Gomori's reticulin, Masson's trichrome, and PAS stains. All tissue was coded and examined by one of us (SR). Control animals, immunized with nonintestinal tissue in complete Freund's adjuvant, were included in a random manner.

Table 1—Liver and Kidney Disease in Rabbits Immunized With Intestinal Antigen

Immunizing agent	Liver disease	Kidney disease
Rabbit duodenum	++	NE
	−	NE
	+++	NE
Rabbit ileum	−	NE
	+	NE
Rabbit colon	−	IN
	−	−
	−	−
	++	IN
	+	−
	−	−
	++	−
	+++	−
	+	IN
	+	IN
	−	−
Germfree rat duodenum	+	IN
	−	−
Germfree rat ileum	++	−

+ = Mild chronic active liver disease (see Results), ++ = moderate degree of chronic active disease (see Results), +++ = severe disease (see Results), − = normal liver or kidney tissue, NE = not examined, IN = interstitial nephritis.

Results

Liver Histology

Sixteen rabbits were immunized with rabbit intestinal extract. The histology of normal rabbit liver is shown in Figures 1 and 2. Nine of the 16 (56%) had abnormal liver histology (Table 1). In all of the abnormal rabbits (Figures 3–7), there was an increased inflammatory infiltrate which was generalized throughout the portal tracts and tended to break through the portal limiting plate. In areas where the infiltrate broke through the portal limiting plate, there were small foci of cell degeneration or necrosis (Figure 3). Although the infiltrate was predominantly lymphocytic, plasma cells and polymorphonuclear leukocytes with a few eosinophils were present. In all but the animals with severe disease, the lobular architecture of the liver was maintained.

Within the parenchyma of the mid-zone and antral regions, aggregates of mononuclear cells were encountered replacing dying cells (Figure 4). These changes affecting the portal areas and parenchyma were patchy but were present in each of the nine livers, regardless of the severity of disease. The disease was graded as mild or moderate in severity if only portal and parenchymal infiltrates were present and the lobular architecture was undisturbed with no extension of the infiltrates from one

lobule to the next. In 2 rabbits with severe disease, the inflammatory infiltrate not only bridged portal areas, sometimes delineating whole lobules, but encompassed small nodules of liver cells in the periportal region (Figure 5). Liver cell loss and reticulin condensation was associated with inflammatory cell extension (Figure 6).

Degenerative changes in rabbit hepatocytes included cell enlargement with homogenization of the cytoplasm, nuclear displacement, and rare nuclear pyknosis or karyorrhexis. Cell ballooning with perinuclear cytoplasmic condensation was not a common finding.

An increase in binuclear and multinuclear liver cells with cytoplasmic eosinophilia was interpreted as indicative of regenerative attempts in areas of damage. Fatty change, cholestasis, and hemosiderosis were not noted nor was ductular damage with a fibrotic response.

Two of three rabbits immunized with duodenum or ileum obtained from germfree rats contracted mild to moderate chronic active liver disease (Table 1). Five of ten rabbits immunized with guinea pig intestine extract had mild to moderate liver disease indistinguishable from that seen when the rabbits were immunized with rabbit intestine.

Seven rabbits were immunized with liver antigen (Table 2). Six had chronic active liver disease identical to that found in the rabbits immunized with intestinal extracts (Figure 7).

Controls

Seven rabbits immunized with nonintestinal antigen (2 with tetanus toxoid, 3 with human skin, and 2 with human synovium) in complete Freund's adjuvant had normal liver and kidney histology. Each of these rabbits had high titers of precipitating antibody to the antigen used for immunization.

Kidney Histology

Kidneys of 23 rabbits were examined. Seven rabbits (30%) (2 immu-

Table 2—Liver and Kidney Disease in Rabbits Immunized With Liver

Immunizing agent	Liver disease	Kidney disease
Rabbit liver	++	−
	+	−
	−	−
	+	−
	+++	−
Guinea pig liver	+	IN
	+	IN

See Table 1 for explanation of symbols.

nized with guinea pig liver, 4 with rabbit colon, and 1 with germfree rat ileum) developed interstitial nephritis (Tables 1 and 2). This was characterized by a patchy, predominantly lymphocytic infiltrate in the region of the corticomedullary junction. Migration of mononuclear cells through the tubular epithelium was commonly found (Figure 8). Nephritis was associated with histologically detectable liver disease in 6 of the 7 rabbits.

Tissue Antigens

Due to the finding of hepatic and renal disease in the animals immunized with intestine, antigens shared by these tissues were studied by double diffusion in gel. Rabbit antiserum to rabbit colon tested against rabbit liver, kidney, and colon showed a shared antigen for colon and liver and another for colon and kidney (Figure 9).

Discussion

Hepatobiliary diseases are a common extraintestinal manifestation in patients with ulcerative colitis and Crohn's disease. Chronic active hepatitis has been reported to coexist with ulcerative colitis with a prevalence varying between 5 and 15%.[11,14] In our study, 55% (16 of 29) of rabbits immunized with intestinal tissue and 86% (6 of 7) of rabbits immunized with liver tissue developed inflammatory changes in the liver. This was characterized by portal infiltrates, intralobular infiltrates, breaching of the limiting plate, and periportal cell necrosis. Damage to bile ducts and ductules was not a feature.

A direct relationship between ulcerative colitis and chronic active hepatitis has been suggested by the beneficial effect on a coexisting chronic active hepatitis which occurs following proctocoloectomy.[14] The pathogenic mechanism which produces the liver disease is unknown. Circulating immune complexes, portal vein bacteremia secondary to gut damage or an autoimmune response elicited by viruses or drugs have been suggested.[2,19-20]

Control rabbits immunized with nonintestinal antigens had no evidence of liver disease histologically. As the control antigens, like the intestinal and liver antigens, elicited production of humoral antibody, circulating immune complexes or a nonspecific effect of the complete Freund's adjuvant do not appear to be related to the pathogenesis of this experimental model of liver disease. We have further shown that precipitating serum antibody to intestinal antigens is not a factor in the inflammatory bowel lesions which are produced in these animals.[15]

Immunization of rabbits with intestinal extracts prepared from rabbits, guinea pigs, or germfree rats leads to antibody production to a non-tissue-

specific antigen.[12,13] The non-tissue-specific antigen is shared by the intestinal epithelial cell cytoplasm and the cytoplasm of hepatocytes. This antigen is not related to bacteria, as it is found in the intestinal epithelial cell cytoplasm of germfree rats.

The intestinal changes found in another group of rabbits has previously been reported.[15] In the current study an equal distribution was found when the rabbits were grouped according to those which had only abnormal liver or intestinal histology and those in which changes in both organs were found. Thus, one abnormality does not predispose to the other, and the immune reactivity of the animal to antigen shared by liver and colon is not the sole factor in determining disease pathogenesis.

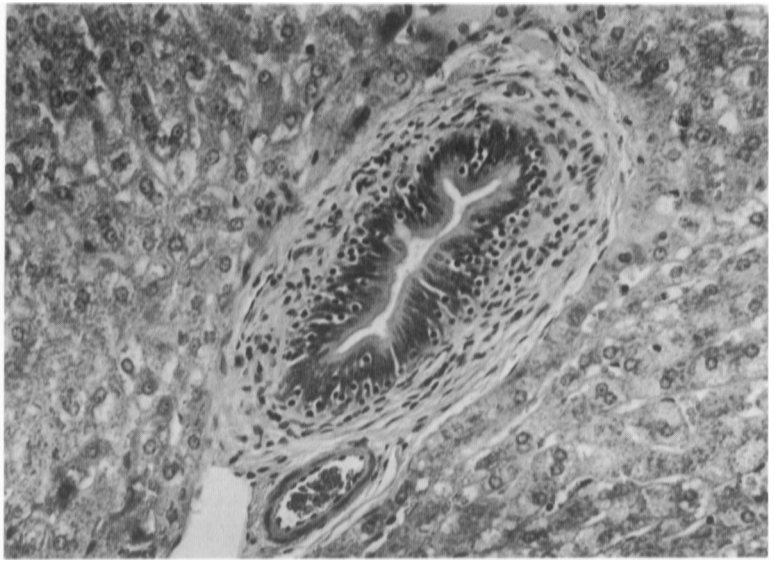
The presence of interstitial nephritis in rabbits injected with liver protein has been noted before in animal models.[7] Seven of 23 animals (30%) injected with gut or liver antigens in this study had interstitial nephritis. Renal tubular acidosis, latent or overt, has been observed in 32% of patients with chronic aggressive hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis.[10,16,17] The histologic picture in these patients is one of a tubulointerstitial lymphocytic infiltrate.[10,16,17] Also, evidence of cellular sensitivity to Tam-Horsfall protein localized in distal tubular and the ascending loop cells has been present in these patients.[18] This, as well as the absence of glomerular lesions in animals and humans, suggests that nonspecific immune complex deposition is not the sole reason of renal dysfunction in chronic liver disease.

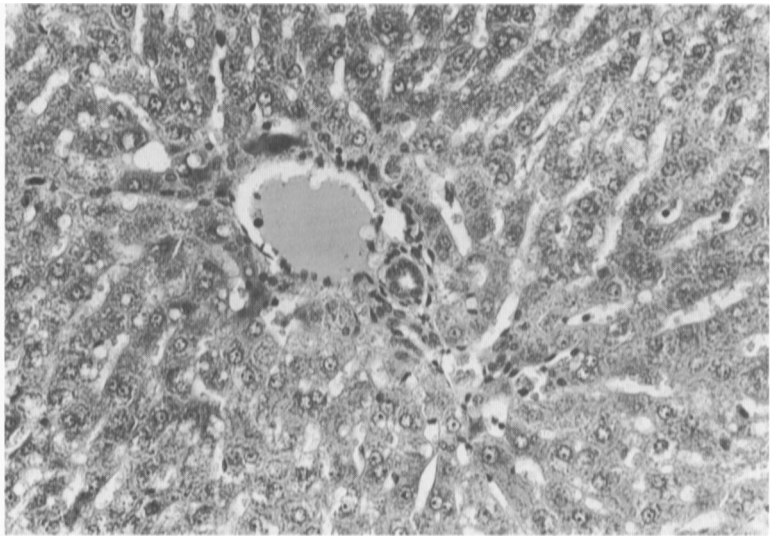
Our data suggest that an immune response to antigens which are common for the intestine, liver, and kidney may be one factor in the pathogenesis of chronic active hepatitis and interstitial nephritis in this experimental system. The common association of inflammatory bowel disease and chronic active hepatitis in man may be due to a similar mechanism.

References

1. Smith MGM, Eddleston ALW, Williams R. Immunological factors in the evolution of active chronic hepatitis and other autoimmune liver diseases. Clin Gastroenterol 4:297–313, 1975
2. Plotz PH: Autoimmunity in hepatitis. Med Clin N Am 59:869–876, 1975
3. Wittingham S, Mackay IR, Irwin J: Autoimmune hepatitis: Immunofluorescence reactions with cytoplasm of smooth muscle and renal glomerular cells. Lancet 1:1333–1335, 1966
4. Paronetto F, Schaffner F, Popper H: Antibodies to cytoplasmic antigens in primary biliary cirrhosis and chronic active hepatitis. J Lab Clin Med 69:979–988, 1967.
5. Smith MGM, Golding PL, Eddleston ALWF, Mitchell CG, Kemp A, Williams R: Cell-mediated immune responses in chronic liver disease. Br Med J 1:527–530, 1972

6. Pipitone V, Albans O: Some aspects of humoral and developed hypersensitivity in chronic hepatitis. Immunology of the Liver. Edited by MGM Smith, R Williams. London, William Heineman Ltd., 1972, pp 204–213
7. Meyer zum Büschenfelde KH, Kössling FK, Miescher PA: Experimental chronic active hepatitis in rabbits following immunization with human liver proteins. Clin Exp Immunol 11:99–108, 1972
8. Meyer zum Büchenfelde KH, Kossling FK: Characterization and isolation of liver specific proteins in different species: Their importance for experimental models and in diagnosis of chronic hepatitis.[6] pp 169–185
9. MacLaurin BP: Induction and transfer of autoimmune periductual fibrosis in inbred rat strains—with evidence for predominantly cell mediated pathogenesis.[6] pp 155–168
10. Golding PL, Smith M, Williams R: Multisystem involvement in chronic liver disease: Studies on the incidence and pathogenesis. Am J Med 55:772–782, 1973
11. Mistilis SP, Blackburn CRB: Active chronic hepatitis Am J Med 48:484–495, 1970
12. Rabin BS: Duodenum-, ileum-, and colon-specific antigens. Int Arch Allergy 50:133–141, 1976
13. Rabin BS: The presence and anatomic location of species and organ specific intestinal antigens. Int Arch Allergy (In press)
14. Olsson R, Hulten L: Concurrrence of ulcerative colitis and chronic active hepatitis: Clinical course and results of colectomy. Scand J Gastroenterol 10:331–335, 1975
15. Rabin BS, Rogers SJ: Nonpathogenicity of antiintestinal antibody in the rabbit. Am J Pathol 83:269–282, 1976
16. Golding PL, Mason ASM: Renal tubular acidosis and autoimmune liver disease. Gut 12:153–157, 1971
17. Golding PL, Brown R, Stuart-Mason A: Studies on multisystem involvement in active chronic hepatitis and primary biliary cirrhosis.[6] pp 194–202
18. Tsantoulas DC, McFarlane IG, Portmann B, Eddleston ALWF, Williams R: Cell-mediated immunity to human Tamm-Horsfall glycoprotein in autoimmune liver disease with renal tubular acidosis. Br Med J 4:491–494, 1974
19. Blackwell JB: The effects on the liver of sensitized rats of intravenous injection of antigen. J Pathol Bacteriol 90:259–268, 1965
20. Redeker AG: Chronic hepatitis. Med Clin N Am 59:863–867, 1975





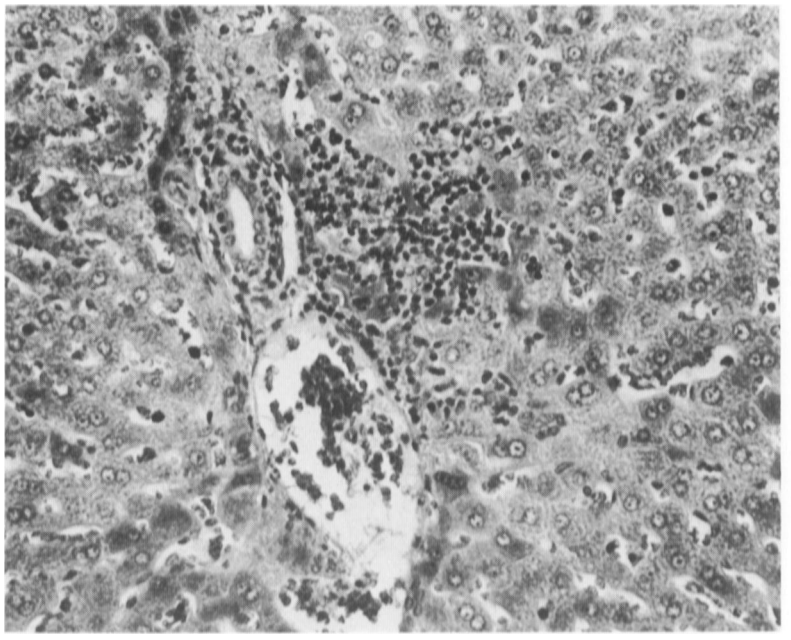
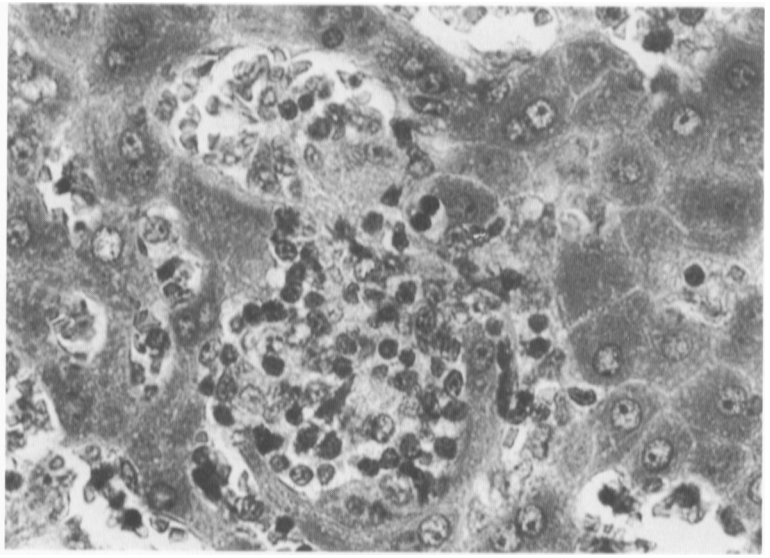
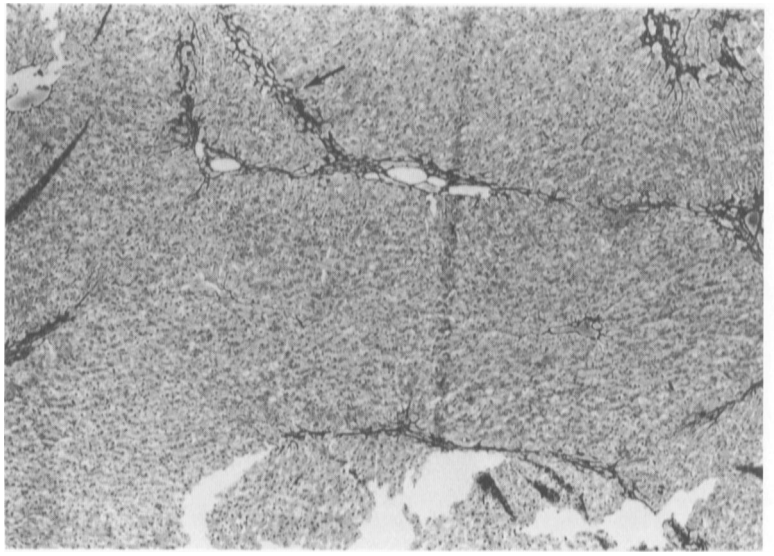


Figure 1—Normal liver with a scanty lymphocytic infiltrate concentrated about the bile duct; interepithelial and intraepithelial lymphocyte migration and dense fibrous tissue cuffing are characteristic. The hepatocyte cytoplasm is speckled. (H&E, × 160) **Figure 2**—A small portal area in normal liver with bile ductules, lymphatic, and a few lymphocytes. There are occasional binucleate cells in the periportal zone (H&E, × 160) **Figure 3**—Colon-immunized rabbit with predominantly lymphocyte infiltrate breaching the portal limiting plate and replacing or surrounding dead and degenerating hepatocytes. Nuclear loss and dark homogenous cytoplasm are features of degenerating cells. (H&E, × 160)

4



6



5

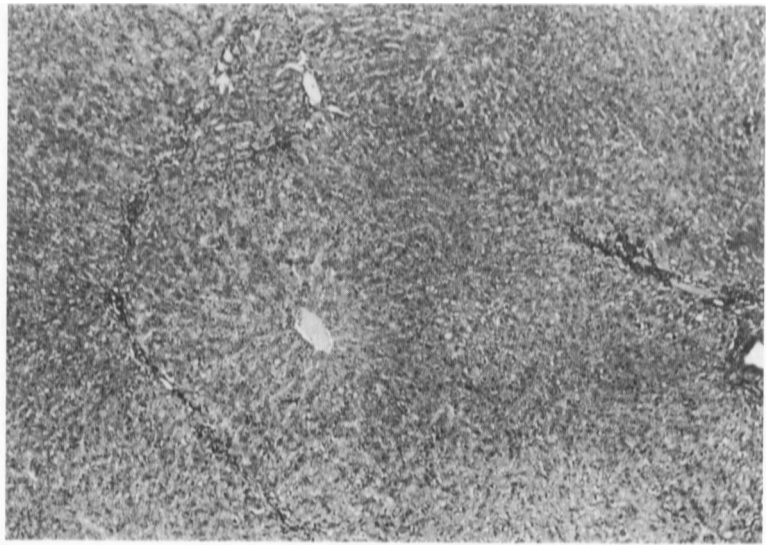
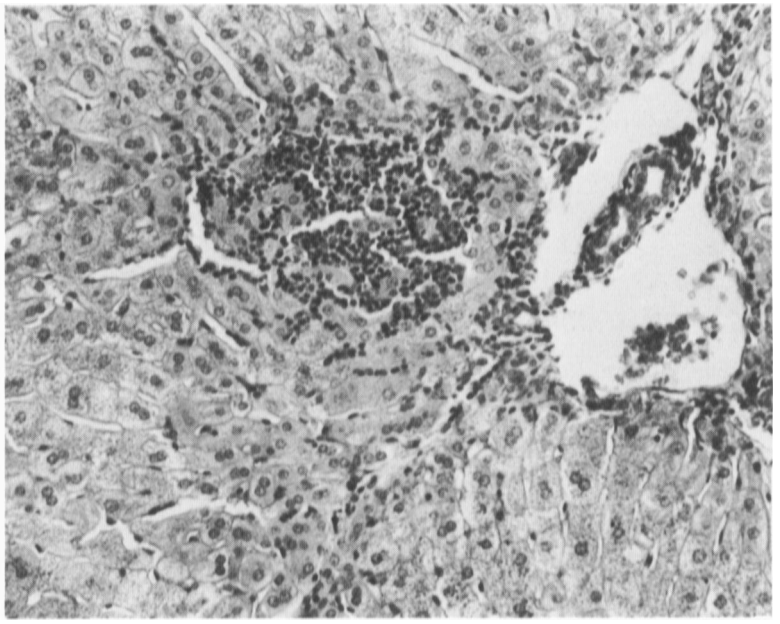
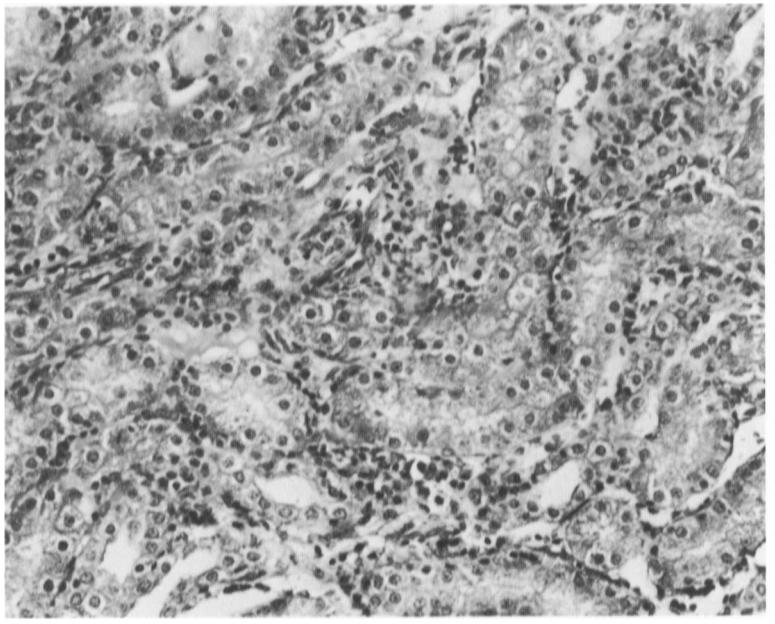


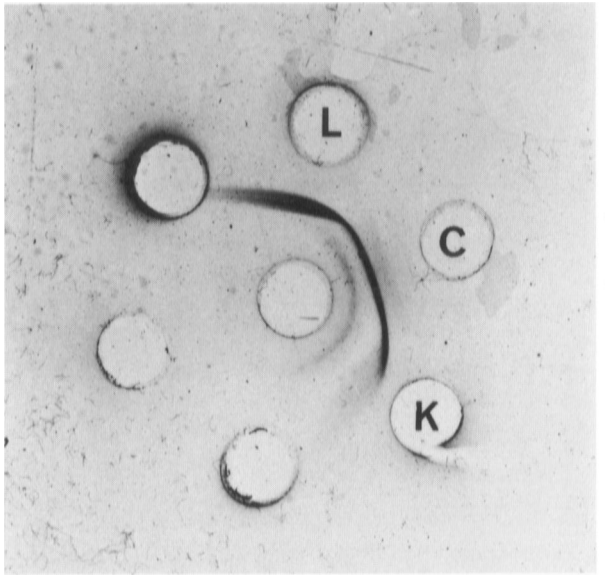
Figure 4—Colon-immunized rabbit with nodule of lymphocytes and hyperplastic Kupffer cells replacing liver cells in the central zone of a liver lobule. Binucleate cells and loss of single cell plates are prominent around the zone of injury. (H&E, × 160) **Figure 5**—Colon-immunized rabbit with the liver lobule outlined by streamers of predominantly lymphocytic infiltrate joining small portal areas. There is mild disarray of hepatocellular cords, and the central vein is slightly eccentric. (H&E, × 40) **Figure 6**—Colon-immunized rabbit with reticulin condensation outlining portions of the lobules as well as a small nodule of hepatocytes (*arrow*). (Gomori reticulin stain, × 40)



7



8



9

Figure 7—Liver-immunized rabbit with pathologic changes similar to those in Figure 4. (H&E, × 160) **Figure 8**—Colon-immunized rabbit with interstitial nephritis in the deep cortex. There is lymphocyte migration into the tubules and sloughing of the epithelium. (H&E, × 160) **Figure 9**—Rabbit antiserum to rabbit colon in center well. Saline extracts of rabbit colon (*C*), liver (*L*), and kidney (*K*) are in the peripheral wells. Both the liver and kidney share antigen with the colon.