Immunocytochemical Reaction of Serum of Patients with Hepatic Diseases With Hepatic Structures.* (26291)

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Preceding studies have indicated the presence of gamma globulin and evidence for its local production in livers near areas of destruction of the parenchyma in hepatitis and cirrhosis(1). This stimulated an investigation as to whether the serum of patients with such diseases, and particularly its gamma globulin fraction contain antibodies for structural elements of the liver. The concept that self perpetuation of hepatic cirrhosis results from an immunologic reaction is old but not established(2). Clinical support comes from cases of lupoid hepatitis described as an antigen-antibody disease(3). Serologic evidences in favor of this are 1) the older investigations showing precipitating antibodies against liver tissue in hepatic disease(4,5) and 2) recent studies with the globulin consumption test(6) and demonstration of complement binding antibodies which, however, are not organ specific(7). Immunocytochemically, in 2 of 7 cases of chronic hepatitis, the serum showed binding to the cytoplasm of liver cells(8).

Material and methods. Sera from 32 patients with various hepatic disease, from 10 patients with lupus erythematosus, and from 30 hospital controls (Table I) were studied by the indirect Coon's technic(9) with cryostat sections of 5 human livers, normal human heart, normal human kidney, rabbit and rat liver, kidney and heart. The sections of human livers (1 normal, 1 malignant hepatitis (rapid transition of viral hepatitis to post necrotic cirrhosis), 1 extrahepatic biliary obstruction, 1 primary biliary cirrhosis and 1 active postnecrotic cirrhosis) were fixed in cold acetone and washed with buffered saline at pH 7. A few drops of serum were placed on each tissue section for 30 min. at room temperature. The sections were then washed with buffered saline for 40 min. Fluoresceinated rabbit antihuman gamma globulin(10) absorbed with liver powder was applied to the sections at room temperature for 30 min. They were washed and mounted in buffered glycerin and examined under the fluorescence microscope. In selected cases gamma globulin was separated from the serum of the patients with a diethylamine-ethylcellulose column(11) and used for comparison with whole serum. In the cases that showed nuclear binding the cryostat sections of liver were incubated with desoxyribonuclease (DNAase) and ribonuclease (RNAase)(12) before application of the serum. The presence of antiblood group substances were determined in all sera. Sera were then absorbed with blood group specific substances A and B and then tested with sections as before.

Results. Binding was encountered to nuclei or to cytoplasm of ductular cells. Nuclear binding (Fig. 1A) was seen with 4 cases of biliary cirrhosis and 2 of malignant hepatitis. It demonstrated all nuclei of all human livers and other human organs as well as of all rat and rabbit organs. It was abolished by pretreatment of the sections with DNAase but not with RNAase. It was present when gamma globulin fractions alone were used instead of serum but was absent when serum minus gamma globulin was applied. In none of the cases was the LE preparation positive. Sera of 2 patients with primary biliary cirrhosis were examined before and after long term corticosteroid therapy. The binding present initially was abolished in one case and diminished in the other. The nuclear binding in lupus erythematosus behaved as that in liver disease except that it did not appear to be influenced by therapy.

Binding to the cytoplasm of ductular cells (Fig. 1B, 1C) was present in 18 cases with various liver diseases and in 1 of 30 hospital controls (Table I). The patient with ob-

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structive jaundice and positive binding had a surgical stricture of the common bile duct for a year and a liver biopsy showed extensive fibrosis and ductular cell proliferation. Binding in the ductular cells was either diffuse or granular, the former being more common, and seems to run parallel with the histochemical PAS reaction of these structures after diastase treatment. Occasionally it marked the contents of the ductular lumen and uniformly the basement membrane. Some sera visualized small ducts at times even more than ductules. Cytoplasm of the hepatic parenchymal cell never was bound. Separated gamma globulin demonstrated ductular cells while

TABLE I. Number of Cases and Type of Binding in Various Liver Diseases, in Lupus Erythematosus and in Control Patients.

Diagnosis	No. of cases	No. with nuclear binding	No. with ductular cell binding
Primary biliary cirrhosis	5	4	2*
Malignant hepatitis	3	2	0
Lupus erythematosus	10	10	0
Viral hepatitis	7	0	5
Laennec's cirrhosis	4	0	4
Postnecrotic "	8	0	6
Juvenile "	2	0	2
Extrahepatic biliary obstruction	3	0	1
Hospital controls without hepatic disease	30	0	1

* 1 only after steroid therapy.

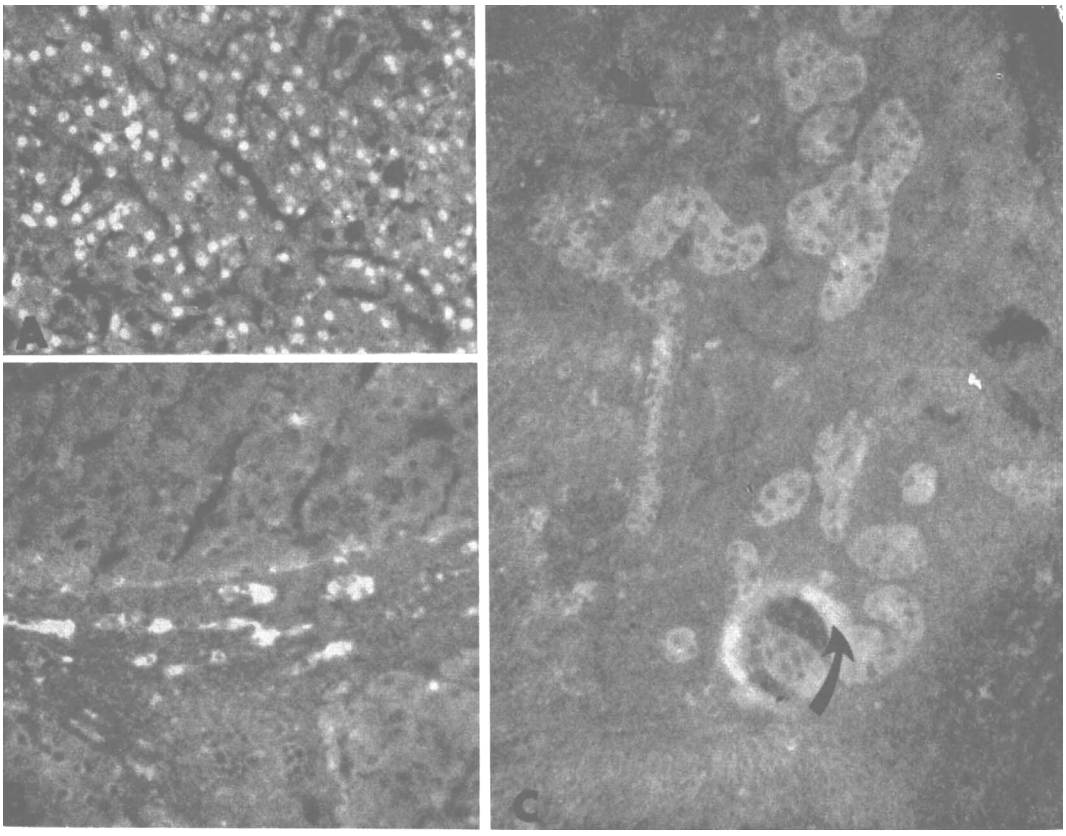

FIG. 1. Fluorescence photomicrographs of cryostat sections of liver after treatment with serum of patients followed by fluoresceinated anti-human gamma globulin antiserum. A. Staining of nuclei of normal human liver by serum of patient with primary biliary cirrhosis (× 250). B. Staining of proliferated ductules of a liver with hepatitis by a serum of a patient with juvenile cirrhosis (× 100). C. Staining of ductules as in (B) by a serum of a patient with chronic hepatitis. Note staining of cytoplasm and not of nuclei and dense staining of bile in the lumen (curved arrow). Straight arrow points to plasmacytoid mesenchymal cells which are also stained by fluoresceinated anti-human gamma globulin without preceding treatment with patient serum (× 250).

serum minus gamma globulin did not. Binding did not correlate with the presence of blood group substances although in some cases it was decreased following absorption of serum with blood group specific substances. Two cases of primary biliary cirrhoses exhibited both nuclear and ductular cell binding, in one simultaneously and in the other after suppression of the nuclear reaction by steroid therapy. Ductular cell binding was most conspicuous with sections of the case of malignant hepatitis and less so in the cases of extrahepatic biliary obstruction and primary biliary cirrhoses. It was not seen in normal liver nor in rat or rabbit liver.

Discussion. Apparently 2 types of reacting substances, presumably antibodies, occur in the serum of patients with liver diseases. One binds nuclei and has neither organ nor species specificity similar to the antinuclear factor found in lupus erythematosus(13,14). However, the cases in question showed no LE phenomena as do cases of lupoid hepatitis, and were either malignant hepatitis or primary biliary cirrhosis. The reaction was abolished or diminished by steroid therapy. Reduction of the reaction by previous treatment with DNAase but not with RNAase suggests that binding occurs with DNA itself or DNA associated proteins as in lupus erythematosus(12). This nuclear binding in liver disease is possibly nonspecific and not pathogenetically related to the disease but may be an anamnestic reaction.

Serum proteins which bind the cytoplasm of the ductules are found in a variety of hepatic diseases with or without nuclear binding. This binding is not influenced by steroid therapy and is directed against material found in a diffuse or sometimes a granular form in the ductular or ductal epithelium and sometimes in the lumen, which means in the bile. Since its appearance parallels granular nonglycogenic PAS positive material in the cytoplasm and in bile, it is either mucopolysaccharide or lipoprotein(15). This is supported by the observation that part of the material seems to be blood group specific substance in nature. Whether it represents an abnormal substance or a retained or aggregated normal substance remains to be established. Even more challenging is the question of a possible pathogenetic role in liver disease of such antigenic material either as a target of an antibody or as an irritating antigen-antibody complex.

Summary. Binding of sera of 32 patients with various liver diseases, 10 patients with lupus erythematosus and 30 hospital controls to human and animal livers and other organs was studied using the indirect Coons' fluorescent antibody technic. Two types of circulating reacting substances were demonstrated: one noted in patients with primary biliary cirrhosis and malignant hepatitis bound nuclei without species or organ specificity; the other occurring in a variety of hepatic diseases reacted with the cytoplasm of ductule cells. The former was influenced by steroid therapy while the latter was not. The antigenic material in the ductular cell possibly is a muco- or lipoprotein which may also be present in bile.

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Evidence of Prenatal Heterosis Relating to X-Ray Induced Congenital Anomalies.* (26292)

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Heterosis or hybrid vigor has been established for adult mice with respect to their tolerance of ionizing radiations (Rugh and Wolff(1). It has also been studied with respect to single organ (testis) reaction where it was found that the resistance of the gonad in the hybrid was more like a simple dominant relation than synergistic heterosis (Rugh, Funk and Wohlfromm(2). Since heterosis can be demonstrated at sexual maturity (2 months of age in the mouse) it seemed pertinent to determine whether it existed prior to birth, and at a time when ionizing radiations quite consistently caused resorptions and congenital anomalies. Thus, this study was designed to establish the resistance of the hybrid to irradiation effects caused by 200 r x-rays at the period of greatest neurogenesis, namely 8.5 days gestation.

Materials and method. The animals used were the CFl Swiss white stock from Carworth Farms, the C57 black/6 stock from Jackson Memorial Laboratory, Bar Harbor, Me., and their F_1 hybrids resulting from the cross between the CFl females and C57 males. This cross is easily made and results in highly viable offspring.

Matings were begun at 5 p.m. The males were removed at 9 a.m. and each exposed female was examined for presence of a vaginal plug. Those with such indications of mating were segregated and at 8.5 days the experimental animals were subjected to 200 r x-rays. The controls, of the same stage of pregnancy, were kept under identical conditions but were not irradiated. At 18.5 days gestation age each of the experimental and control females was killed by cervical dislocation, the uteri were immediately excised, and each fetus, or implantation site, was dissected out and examined. In this way, every successfully fertilized egg which reached the uterus and established an implantation site (at 4.5 days) could be included in the total counts. Each of some 2630 implantation sites in 302 pregnancies was counted as to whether it was normal, resorbed, dead, or showed anomalous development. The anomalies included stunting, exencephaly, anencephaly, protruding intestine, and prominent eye defects. The "normal" mice were so designated when, from all evidence, they appeared normal as to size, development, and absence of obvious anomaly. Mice at 18.5 days are essentially at the delivery stage of development (normally 20-21 days) but if allowed to reach delivery, the mother would generally kill and eat those which are abnormal. Thus, by studying the fetuses *in utero* we are able to present data on total irradiation effect.

The irradiation facilities used included a single x-ray tube at 48.5 cm distance to the position of the gravid uterus, the radiation interrupted by 0.28 mm Cu. and 0.50 mm Al. filters so that the output in air at the level of the 8.5 day embryos was 50 r/minute. Since the mouse is relatively small and the embryos are close to the body surface, extrapolation to rads is well within the maximum range of 3%. The total dose was 200 r in 4 minutes.

Experimental data. Table I presents an adequate number of pregnancies and implantations for statistical significance. Although

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