Experimental Alcoholic Hepatitis: A New Primate Model

Abstract. *Research into the pathogenesis of alcoholic cirrhosis has suffered from the lack of an animal model of alcoholic hepatitis, considered by many to be the link between alcoholic fatty liver and cirrhosis. The entire constellation of histologic features characteristic of alcoholic hepatitis has been produced for the first time in baboons by administration of ethanol with a nutritionally adequate diet. This includes fat, necrosis, inflammation, alcoholic hyaline, fibrosis, and central hyaline sclerosis.*

Chronic alcoholics develop three major types of liver disease, namely, fatty liver, alcoholic hepatitis, and cirrhosis (*1*). Whereas fatty liver is always reversible upon discontinuation of excess alcohol (ethanol) consumption, alcoholic hepatitis may progress to cirrhosis, or may prove fatal in the acute stage. The liver in alcoholic hepatitis exhibits characteristic histologic features (*2*), including fat, ballooning of liver cells, Mallory's alcoholic hyaline, polymorphonuclear leukocytes, diffuse fibrosis, and a necrotizing and fibrotic process surrounding central veins, known as sclerosing hyaline necrosis (*3*). While fat is common, its presence is not necessary for the diagnosis of alcoholic hepatitis. On the other hand necrosis and inflammation appear to be crucial. This disorder has been thought by many to be the link between fatty liver and cirrhosis.

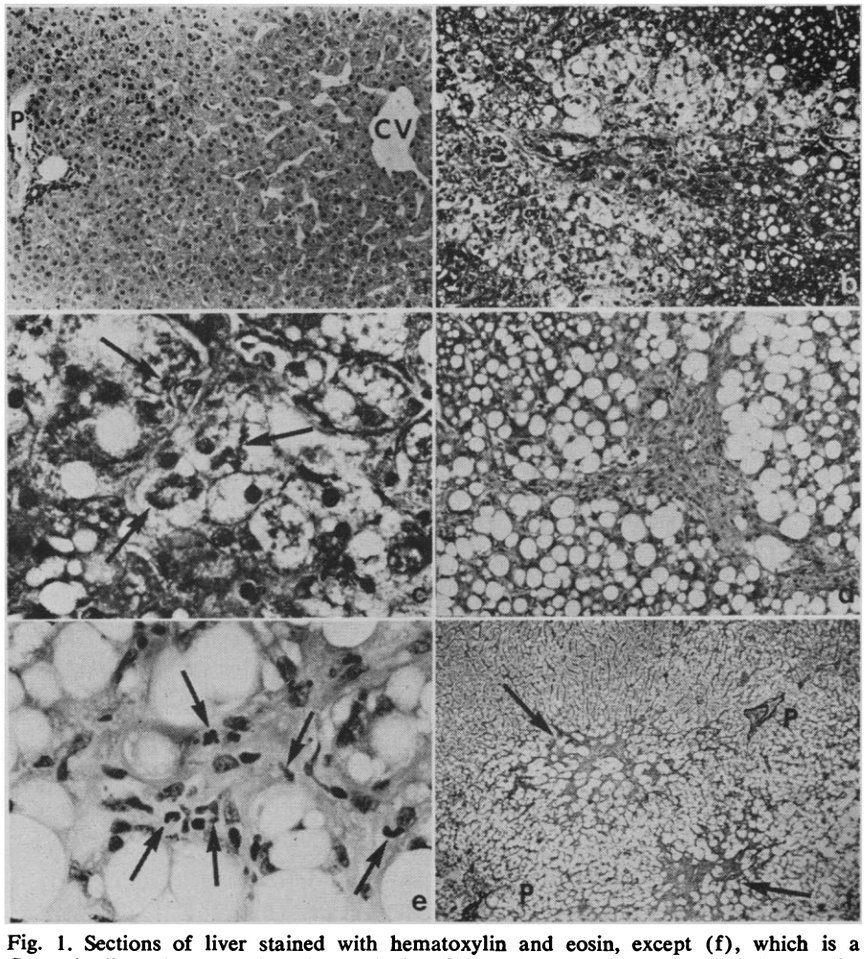

Fig. 1. Sections of liver stained with hematoxylin and eosin, except (f), which is a Gomori silver impregnation for reticulin fibers. (a) Control animal fed the diet without ethanol. The appearance of the liver is normal; *P*, portal tract; *CV*, central vein (× 145). (b through f) Livers from baboons fed ethanol as an isocaloric substitute for carbohydrate for 9 months. (b) Centrolobular zone showing fat accumulation and ballooning of liver cells. The central vein is obliterated (× 145). (c) High-power view of centrolobular cells seen in (b). Arrows point to alcoholic hyaline (× 565). (d) Centrolobular zone showing severe fat accumulation and an area of central hyaline sclerosis, which contains fibrous tissue and numerous inflammatory cells (× 145). (e) High-power view of central hyaline sclerosis shows the presence of mononuclear inflammatory cells and polymorphonuclear leucocytes (arrows) (× 565). (f) A stain for reticulin fibers shows severe sclerosis of the central zones (arrows); *P*, portal tract (× 56).

Experimentally, chronic ethanol ingestion has been shown to produce fatty liver and ultrastructural changes in rats (*4*) and human volunteers (*5*), independent of nutritional factors. Further investigation of the transition from fatty liver to cirrhosis has been hampered by the lack of a suitable animal model for alcoholic hepatitis. Since, in the rat, administration of ethanol with an adequate diet has produced fatty liver without necrosis, inflammation, or fibrosis, we performed experiments with the baboon, whose metabolism is presumably closer to that of man.

The experimental group consisted of six adolescent female baboons, weighing from 8.5 to 11 kg. Three were fed for 9 months a totally liquid, nutritionally adequate diet, in which protein comprised 17, fat 22, and carbohydrate 61 percent of total calories. This diet was supplemented with excess vitamins and minerals (*6*). The other three animals were pair fed the same diet, except that ethanol isocalorically replaced carbohydrate, accounting for 50 percent of total calories. The amount of ethanol consumed daily ranged from 5.6 to 6.7 g/kg.

After 9 months, the activity of serum glutamic oxalacetic transaminase (SGOT) was 40, 37, and 35 units per milliliter in control animals, compared with 2650, 227, and 123 in baboons fed ethanol. While values for SGOT activity between 100 and 300 units are common in alcoholic hepatitis, the value of 2650 is considerably higher than that usually found. This single high value may be related to the difference in species. Serum bilirubin and alkaline phosphatase activity were unchanged by alcohol feeding. At laparotomy, the livers of control animals appeared normal grossly and microscopically (Fig. 1). By contrast, the liver in all biopsy specimens from chronically intoxicated animals showed severe abnormalities (Fig. 1). These included fat accumulation and numerous foci of mononuclear inflammatory cells, intermixed with scattered polymorphonuclear leukocytes throughout the lobules, but most striking in the central zones. Of the hepatocytes which did not contain large fat globules, many exhibited a swollen pale cytoplasm. In one animal, such cells also contained irregular, paranuclear, cytoplasmic inclusions, which appeared identical with alcoholic hyaline seen in human alcoholic hepatitis. These inclusions were purplish-red in sections

stained with hematoxylin and eosin, and blue after staining with aniline blue. The irregular, skeinlike appearance of the hyaline bodies clearly differentiated them from giant mitochondria, which are occasionally visible by light microscopy as regular, circumscribed, round, or ovoid cytoplasmic globules (*1*). Necrotic zones, containing numerous mononuclear and occasional polymorphonuclear inflammatory cells, were frequent around central veins. These areas showed conspicuous fibrosis, which occasionally obliterated the efferent veins. In some areas, thin fibrous septa connected adjacent central zones.

The entire constellation of histologic features characteristic of human alcoholic hepatitis has been produced for the first time in an experimental model. Moreover, the activity of serum transaminase was also increased, a common finding in alcoholic hepatitis. On the basis of early experiments with rats fed ethanol in drinking water (*7*), it was claimed by some that liver disease related to alcohol abuse is caused by associated malnutrition, and that a nutritionally fortified diet protects against the deleterious effects of chronic alcoholism. The data presented here indicate that not only fatty liver, but also alcoholic hepatitis, is a direct result of alcohol toxicity, and is not related to nutritional factors, since the diets of all animals contained excess protein, minerals, and vitamins. In man, alcohol-induced cirrhosis of the liver, often preceded by alcoholic hepatitis, usually follows 10 to 15 years of excess alcohol consumption (*8*). It is, therefore, not surprising that this irreversible phase of liver disease was not seen after only 9 months. It will be of interest to see whether the baboons eventually develop cirrhosis.

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6. The animals consumed daily 78 to 94 mg/kg of a diet which contained the following ingredients per liter: *dl*-α-tocopherol acetate, 30 mg; thiamine hydrochloride, 1 mg; pyridoxine hydrochloride, 1 mg; folic acid, 1 mg; calcium pantothenate, 3 mg; nicotinic acid, 5 mg; ascorbic acid, 25 mg; choline chloride, 100 mg; inositol, 100 mg; *p*-aminobenzoic acid, 100 mg; vitamin B_{12}, 1 μg; vitamin A, 4000 international units (I.U.); vitamin D_3, 400 I.U.
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Antibodies to Herpesvirus Nonvirion Antigens in Squamous Carcinomas

Abstract. *Serums from tumor-bearing patients, cured patients, and normal subjects were examined for antibodies to the separated complement-fixing reactive components of nonvirion antigens of herpesvirus type 1 and type 2. The occurrence of antibodies to the antigens was similar in serums from tumor-bearing patients and cured patients. Antibodies to the antigens were observed among 21 of 24 (87 percent) cervical cancer cases, 44 of 49 (90 percent) laryngeal cancer cases, 15 of 24 (62 percent) cases of squamous cell carcinomas of the head and neck excluding the larynx, 2 of 24 (8 percent) nonsquamous cell cancer cases, and 3 of 51 (6 percent) normal subjects. By contrast, no differences were found in the titers of neutralizing antibodies to the virus in serums from laryngeal cancer patients and controls. The observations support an etiologic role of herpesviruses in cervical cancer and in laryngeal cancer, and possibly other squamous cell cancers of the head and neck.*

Concentrates of purified antigens from viable cells from squamous cell carcinomas of the genital tract reacted in complement-fixation tests with antibodies present in the serums of patients with squamous cell cancers of the same site (*1*). With DNA tumor viruses, it has been possible to separate virus-induced, nonvirion antigens not only from tumors but also from cells undergoing lytic infection by the viruses (*2*). Separated antigens from squamous cell carcinomas of both the lip and cervix react specifically with antibodies to herpesvirus (HSV) nonvirion antigens (*3*). Antibodies to HSV nonvirion antigens have been detected in serums from patients with cervical cancer (*3, 4*), as well as patients with cancers of the lip (*3*), prostate, urinary bladder, kidney, and nasopharynx (*5*). Our study was undertaken to find out whether serums from patients with squamous cell cancers at various sites and with nonsquamous cell cancers contained antibodies to HSV nonvirion antigens. In addition, the influence of successful therapy on the presence of antibodies to the above antigens was evaluated.

The tumor antigens were separated by a special method of polyacrylamide gel electrophoresis (PAGE) into three gel regions; cancer gel region 3, near the anodal end of the gels, was positive for complement-fixing (CF) reactivity with the antiserums specific for HSV nonvirion antigens (*6*). With recent techniques of sequential, stepwise, low-frequency sonication, and subsequent centrifugation, the soluble portion of HSV nonvirion antigens has been studied with several strains of the virus (*7*). Analysis by PAGE of each sonicated fraction reveals the slow appearance of the genetic marker; after the final sonication all of the CF reactivity is present in the supernatant, with the final pellet being negative for CF reactivity (*7*). The portion of the supernatant separated by PAGE, which

Table 1. Comparison of antibodies to herpesvirus nonvirion (by complement fixation) and virion (by neutralization) antigens among patients with carcinoma of the larynx and normal persons.

Group	Tested (No.)	Mean age (years)	Antibodies to herpesvirus			
			Neutralization*		Positive CF†	
			Type 1	Type 2	No.	Percent
Carcinomas of larynx‡	38	59.5	2.17§	1.75§	36	95
Controls	36	58	2.19	1.82	2	5

* Mean titers of neutralizing antibodies are as $\log_{10}$. One case and one control had no neutralizing antibodies to either virus. † Reactions in complement-fixation test to type 1 or type 2 (or both) nonvirion antigen. ‡ 91 percent nonvirion antigen-positive serums from tumor-bearing and 100 percent nonvirion antigen-positive serums from patients up to 4 years after treatment. Primary sites were 21 percent supraglottic and 79 percent glottic. § Data from (*8*).