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Sequential Production of Fatty Liver, Hepatitis, and Cirrhosis in Sub-Human Primates Fed Ethanol with Adequate Diets

(alcoholism/fibrosis/microsomes/mitochondria/liquid diets)

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ABSTRACT This study reproduces in experimental animals the sequential development of all the liver lesions seen in the human alcoholic: in 15 baboons fed ethanol, all developed fatty liver, five progressed to hepatitis, and five had cirrhosis. Maintenance of a nutritionally adequate regimen despite the intake of isocaloric amounts of ethanol (50% of total calories) was achieved by incorporation of the ethanol in a totally liquid diet. Upon ethanol withdrawal, signs of physical dependence, such as seizures and tremors, developed. Ultrastructural changes of the mitochondria and the endoplasmic reticulum were already present at the fatty liver stage and persisted throughout the hepatitis and cirrhosis. The lesions were similar to those observed in alcoholics (including the inflammation and the central sclerosis) and differed from the alterations produced by choline and protein deficiencies. At the fatty liver stage, some "adaptive" increases in activity of microsomal enzymes [aniline hydroxylase (EC 1.14.14.1) and the microsomal ethanol oxidizing system] were observed, but these tended to disappear with the development of hepatitis and cirrhosis. Fat accumulation was also much more pronounced in the animals with the hepatitis as compared with those with simple fatty liver (an 18-fold compared with 3- to 4-fold increase in liver triglycerides). The demonstration that these lesions can develop despite an adequate diet indicates that in addition to correction of the nutritional status, control of alcohol intake is mandatory for the management of patients with alcoholic liver injury.

With increasing alcohol consumption, the incidence of related complications has been rising steadily, particularly that of associated liver disease, to the extent that at the present time, cirrhosis of the liver, the most severe hepatic complication of alcoholism, is the third cause of all deaths between the ages of 25 and 65 in the city of New York (1). In addition to cirrhosis of the liver characterized by diffuse hepatic scarring, alcohol abuse is also associated with hepatic inflammation and necrosis (alcoholic hepatitis) and excess fat accumulation (alcoholic fatty liver). The relationship of these various liver injuries to each other, however, has been questioned. Furthermore, since not all alcoholics develop liver injury, there has been considerable debate concerning the question whether alcohol itself or some associated factor, such as dietary deficiency, is the main cause for the liver disease. The question has both theoretical and practical implications. The classic belief that liver injury could be prevented in the alcoholic by merely controlling the diet was challenged by evidence that alcohol might exert direct toxic effects upon the liver; it was indeed shown that the fatty liver, the most benign stage of the disease, could be produced in volunteers given alcohol in association with adequate or enriched diets (2-4). Fatty

liver, however, is still a fully reversible lesion and the question remained whether alcoholic hepatitis, associated with a high morbidity, and irreversible cirrhosis could also be linked directly to alcohol ingestion itself rather than to a deficient diet. This problem could not be studied in volunteers, in view of the severity of the lesions involved. Previous attempts to produce these lesions in animals failed because of the reluctance of all species used to consume enough alcohol when the latter was given as part of the drinking water. This natural aversion for ethanol was overcome by the incorporation of ethanol in totally liquid diets. This new experimental model for alcohol feeding showed that even when given with adequate diets, ethanol can cause alcoholic hepatitis and cirrhosis in nonhuman primates. The development of this new experimental model clarifies the question of the etiology of liver injury associated with alcohol abuse and represents a new tool for the development of rational forms of prophylaxis and therapy.

MATERIALS AND METHODS

The detailed composition of the liquid diet fed to the baboons is given elsewhere (5). The protein content (18% of total calories) corresponds to that of commonly used commercial diets that are satisfactory for the baboon and is almost twice the amount recommended for human diets (6). The mineral and vitamin content of the diet exceeded the requirements formulated for the monkey (7-9). Its caloric value was 1 calorie/ml. The diet was prepared by Bio Serv Inc., Frenchtown, N.J., and was given to the baboons twice a day in standard drinking bottles equipped with an outlet valve. Each alcohol-fed animal was matched with a control, the dietary intake of which was identical except for the isocaloric substitution of carbohydrate by ethanol to the extent of 50% of total calories. This technique of daily pair feeding was adopted to assure a strictly equal caloric intake in both ethanol-treated animals and in their individual pair-fed controls.

The 30 adolescent or young animals used for this study were either *Papio hamadryas* or olive and yellow baboons. Twelve animals were raised in this country, whereas the remainder were imported from Africa and were studied after prolonged quarantine periods. They were housed in individual cages at the Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP), Tuxedo, N.Y. Until the actual study period, they were given a routine regimen of Purina® monkey chow *ad libitum*, supplemented with a daily vitamin prep-

cirrhosis (severe and irreversible scarring of the liver). To comprehend fully the significance of this article, it is helpful to have a good historical perspective on the controversy that dominated the field at the time it was published. At the center of the controversy was this question: Does alcohol itself or do other factors such as dietary deficiency cause ALD? Not only was this issue scientifically important, but it also had serious practical implications.

Because patients with ALD often have accompanying malnutrition, deficiencies of key nutrients were thought to be a primary cause of this disease. This view was exemplified by the early studies of Best and colleagues (1949) and Koch and colleagues (1969), which demonstrated that alcohol-induced fatty liver could be prevented in rats when the animals' diets were supplemented with choline or protein. Lieber and colleagues challenged this theory even though it was widely supported by the scientific community.

In their study, Lieber and colleagues fed a liquid diet containing alcohol that was originally developed for rats to a species related more closely to humans—the baboon. The researchers chose baboons because an earlier study had shown that, as a result of their aversion to alcohol, rats fed this liquid diet derived no more than 36 percent of their total calories from alcohol and failed to reproduce liver disease beyond fatty liver. Lieber and colleagues hypothesized that baboons might overcome this limiting factor and consume enough alcohol to induce more advanced ALD. Indeed, their study demonstrated that the baboons' alcohol consumption reached

50 percent of their total calories, a percentage approximating the caloric amount that many human alcoholics derive from alcohol. In addition, these animals exhibited the sequential development of the whole spectrum of ALD, ranging from fatty liver and hepatitis to cirrhosis. Because the liquid diet was designed to provide adequate nutrition, the baboons' development of liver disease was interpreted as evidence supporting alcohol's causal role in the development of ALD.

Epidemiological studies of alcoholic patients by Lebach (1975) and Pequignot and colleagues (1978) supported this conclusion, showing that the risk of developing alcoholic liver cirrhosis increases in proportion to alcohol consumption. These results implied that to manage patients with ALD successfully, alcohol intake must be controlled and that nutritional support alone might not prevent the development of the disease.

Lieber, C.S.; DeCarli, L.M.; and Rubin, E. Sequential production of fatty liver, hepatitis, and cirrhosis in sub-human primates fed ethanol with adequate diets. Proceedings of the National Academy of Sciences USA 72(2):437-441, 1975.

FATTY LIVER, HEPATITIS, AND CIRRHOSIS IN SUB-HUMAN PRIMATES FED ETHANOL

Commentary by Hidekazu Tsukamoto, Ph.D.

KEY WORDS: alcoholic liver disorder; fatty liver; liver cirrhosis; hepatitis virus, AOD effects (AODE); animal model; diet; nutritional deficit

This study by Lieber and colleagues published in 1975 is one of a few seminal articles written in the past 25 years that has made a critical impact on the subsequent direction of research conducted on the mechanism of alcoholic liver disease (ALD), which usually progresses from fatty liver to hepatitis (inflamed liver with dead liver tissue) to liver fibrosis (scarring liver) to

Over the past two decades, Lieber and colleagues' findings indicating that alcohol is a primary causative agent for ALD inspired more aggressive studies on the biochemical basis for alcohol's injurious effects on the liver. As a result, several hypotheses emerged, all of which linked the chemical breakdown of alcohol (i.e., metabolism) to the development of ALD. Alcohol metabolism generates harmful chemical products and byproducts (e.g., acetaldehyde [a metabolic product of alcohol] and free radicals [highly reactive molecules]) and creates deleterious metabolic and physiologic conditions that make the liver more susceptible to injury. Lieber continues to play a leadership role in much of this research.

Lieber and colleagues' article described the first animal model for the spectrum of progressive and advanced ALD. The baboon model offered great potential for investigations examining the biochemistry, cell biology, and molecular biology of ALD. Popper and Lieber (1980) further clarified the progression of ALD in this animal model by meticulously examining liver specimens taken from the baboons at each stage of their disease. They revealed that evidence for the development of alcoholic hepatitis in baboons was lacking but that liver fibrosis and cirrhosis were developing directly from fatty liver. This was a provocative and significant finding for the understanding of alcoholic liver fibrosis development, because it indicated that liver fibrosis could be induced without death of liver cells and inflammation. These changes had long been considered important signals for the onset of liver fibrogenesis. This finding was confirmed by later studies showing the similar evolution of liver fibrosis in alcoholic patients.

Further research by Lieber demonstrated the following:

- Fibrosis surrounding a vein in the middle of a small anatomical unit of the liver (central vein) signals the beginning of progressive alcoholic liver fibrosis
- Transformation of the liver cells that store vitamin A (hepatic stellate cells) to the cells involved in scarring (myofibroblasts) is the cellular basis of the development of alcoholic liver fibrosis
- The role of acetaldehyde in stimulating production of fibrous proteins by hepatic stellate cells
- The effectiveness of using polyunsaturated lecithin (a type of lipid in the cell wall) as a therapy in alcoholic liver cirrhosis.

Most of these findings would not have been possible without the baboon model.

Although the baboon model has produced an enormous volume of new information concerning the mechanism of ALD, this model has not been reproduced by others (Rogers et al. 1981; Mezey et al. 1983). The possibility exists that the differences in the species (e.g., in later studies, rhesus monkeys were used as models in place of baboons) or variations in the feeding techniques might have resulted in the failure to reproduce ALD. The reproduction of the baboon model by independent investigators undoubtedly would help promote the appreciation and the wide use of the model. Emerging evidence from studies using the baboon and other animal models clearly points to how nutritional factors—both in deficiency and in excess—can sensitize the liver to the deleterious effects of alcohol and its metabolism. Use of the baboon model by different and independent laboratories may help address this issue in future research. ■

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