PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 370–374 (1975)

Cirrhosis of Choline Deficiency in the Rhesus Monkey. Possible Role of Dietary Cholesterol (38541)

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Although cirrhosis has been produced in the rat by choline-deficient diets it has been questioned whether the primate is susceptible to this disease. Earlier attempts to produce cirrhosis in baboons (1) and in cebus and rhesus monkeys (2) were unsuccessful. It was suggested that the low choline oxidase of primates may indicate less need of dietary choline (3).

However, in 1959 Wilgram (4) reported the production of a Laennec-type cirrhosis in two cebus monkeys on a choline-deficient diet. Subsequent reports (5–9) confirmed this finding with the rhesus monkey by means of the Wilgram diet. This contained 10% peanut meal, 5% soya protein, 18% lard, 62% sucrose–starch–dextrin, vitamins, salts, and 2% cholesterol. Although the diet was deficient in choline the cirrhogenic effect could have been due in part to other factors. Peanut meal might possibly have been contaminated by aflatoxins, to which the rhesus monkey is highly susceptible (10). The supplement of dietary cholesterol may have exerted an hepatotoxic effect. It has long been known that dietary cholesterol can cause fatty liver (11, 12), hepatocellular injury and cirrhosis in rabbits and rats (13–16). Therefore, it seemed desirable to determine whether cirrhosis could be produced in the monkey by a choline-deficient diet lacking these factors. The diet employed has produced cirrhosis with regularity in the rat (17). In this diet, casein was the source of protein.

Methods. Eighteen rhesus monkeys (Macacca mulatta) approximately 2 yr old, were placed two in a cage in an air-conditioned room. The cages had wide meshed floors which prevented feces from accumulating. The animals were assigned to one of three dietary groups: Group I (10 monkeys) was fed the basic choline-deficient diet; group II (five monkeys) the same diet with added choline; group III (three monkeys) commercial chow. After 14 mo the 10 animals in group I had shown no significant changes in liver biopsies, and they were then divided into two subgroups: Five monkeys (group Ia) were maintained on the original diet; five monkeys (group Ib) received a cholesterol supplement comprising 2% of the diet. The experiment was continued for an additional 17 mo for a total of 31 mo.

Table I shows the composition of the diets, which were fed *ad libitum*. Needle biopsies of the liver were obtained at 3-mo intervals. Prior to biopsy venous blood was drawn for hematocrit, hemoglobin, and chemical determinations, which included serum bilirubin, SGOT, alkaline phosphatase, lactic acid dehydrogenase, total proteins, albumin, electrolytes, calcium, phosphorus, blood urea nitrogen, uric acid, glucose, and cholesterol. Paraffin sections of liver were prepared with hematoxylin and eosin, Sudan, trichrome, and iron stains. Representative sections were processed for electron microscopy.

Results. All animals were vigorous except for two that developed cirrhosis. Growth rates were similar for experimental groups and chow-fed controls. None died prematurely. Patchy alopecia developed in several monkeys fed the cholesterol supplement. The only noteworthy changes were a moderate anemia and hypoalbuminemia in the protein- and choline-deficient animals. Blood chemistries were otherwise normal except for the two animals developing cirrhosis.

Livers of animals in groups I and II showed minor changes on light microscopy, such as fine vacuolation and ballooning of hepatocytes. There were slight fatty changes, usually visible only with Sudan stain. A sharp increase in fat was seen in livers of five monkeys fed supplemental cholesterol

TABLE I. EXPERIMENTAL DIETS FED DIFFERENT GROUPS OF MONKEYS
g/100 g.

	Ia	Ib	II	III
Casein, vitamin-free	5.0	5.0	5.0	Monkey
Sucrose	60.5	58.5	60.0	Chow
Corn starch	22.0	22.0	22.0	Ralston
Corn oil	8.0	8.0	8.0	
Salt mix, Hegsted IV	4.0	4.0	4.0	
L-Cystine	0.5	0.5	0.5	
Vitamin mix[a]				
Choline chloride	—	—	0.5	
Cholesterol	—	2.0		

[a] Vitamin mix: After 6 mo there were no significant hepatic changes. It was felt that vitamin supplements may have been rich in choline precursors. The mixture was changed for the ensuing 24 mo to the following, in g/kg diet: PABA 0.0; ascorbic acid 0.50; biotin 0.0002; B_{12} 0.00005; Ca pantothenate 0.0025; folic acid 0.001; inositol 0.5; menadione 0.0005; nicotinic acid 0.04; pyridoxine HCl 0.004; riboflavin 0.008; thiamin HCl 0.004.

Vitamin A palmitate	20,000	IU/kg diet
Vitamin D_3	2,000	IU/kg diet
Vitamin E acetate	52.4	IU/kg diet

(group Ib). Chow fed animals had normal livers throughout.

Cirrhosis developed in one monkey of group I after 26 mo on the choline-deficient diet. The animal became listless and icteric. Serum bilirubin rose to 4.3 mg. percent, SGOT to 244 K units, serum albumin fell to 1.7 g/100 ml, hematocrit 27, Hgb 8.4 g. Previous laboratory values had been normal except for moderate hypoalbuminemia. The elevated serum bilirubin and SGOT subsided in 2 mo although the diet was unchanged. Cirrhosis progressed in the ensuing 5 mo.

The second case occurred in a monkey fed the same choline-deficient diet for 14 mo, at which time the liver appeared normal on biopsy. Cholesterol then was added to the diet. Two months later, liver biopsy revealed fatty changes, scattered foci of necrosis, and moderate periportal fibrosis with intralobular extension. Subsequent biopsies showed the progressive nodularity of cirrhosis. A

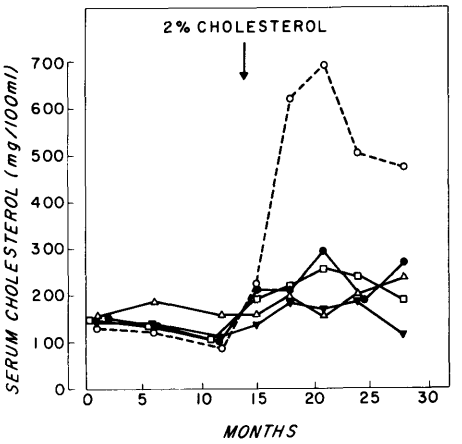

FIG. 1. Serum cholesterol in five monkeys fed choline deficient diet with cholesterol supplement.

sharp rise in SGOT occurred 2 mo after cholesterol supplementation. This subsided after 3 mo, although the diet was unchanged. There was no increase in serum bilirubin. This monkey had the most marked and sustained rise in serum cholesterol of the five animals fed this supplement (Fig. 1).

Figures 2 and 3 illustrate histological changes in the two cirrhotic monkeys.

Electron microscopy showed depletion of cytoplasmic constituents in those fed low-protein diets, especially of endoplasmic reticulum, but it was otherwise unremarkable. Sequential biopsies in one animal developing cirrhosis gave no indication of impending changes.

The study was terminated at 31 mo. There was no hepatitis nor hepatic disease in the primate colony at the time of the experiments.

Discussion. In the present study rhesus monkeys fed a choline-deficient diet showed less susceptibility to cirrhosis than those observed by Wilgram (4) and by others (5–9) employing his diet. There are several possible causes for the disparate results, such as differences in diet, in animal susceptibility, or possible contamination with aflatoxins or other toxins. The explanation does not appear to relate to the degree of choline deficiency, since upon analysis[1] the Wilgram

[1] Analysis of the diets for content of choline and methionine was made by Warf Institute, Madison, WI.

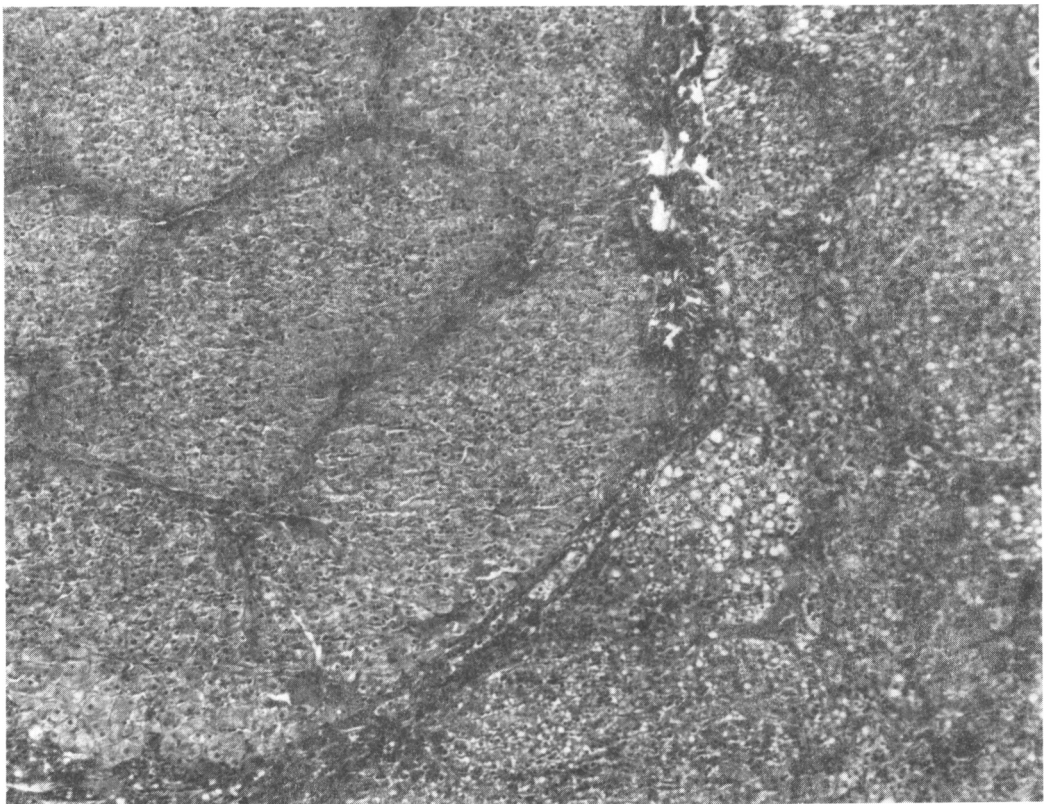

FIG. 2. Liver section from monkey in group I, showing fibrosis, pseudolobulation, and fatty changes. H and E ×40.

diet contained more choline and methionine than did our diet. Although contamination with aflatoxins in peanut meal was possible, the lesions associated with aflatoxin toxicity (such as hemorrhagic necrosis and bile duct proliferation) were not encountered. It seems more plausible that dietary cholesterol exerted an hepatotoxic effect. The abrupt rise of liver lipids in five monkeys after cholesterol supplementation supports this interpretation. As stated earlier there is considerable evidence that dietary cholesterol can exert hepatotoxic effects in rabbits and rats (11–16) particularly in the presence of choline deficiency.

The Wilgram diet provoked a greater rise in serum cholesterol than seen in our monkeys fed the same cholesterol supplement (group 1b). This probably was related to differences in the fat contents of the diets. Maximal absorption of cholesterol occurs with diets containing 15–25% fat. The Wilgram diet contained 18% fat whereas ours contained 8% fat.

Clarkson and his associates (18) observed that plasma levels of cholesterol vary considerably in squirrel monkeys fed the same intake of cholesterol. Some developed severe hypercholesterolemia (hyper - responders) whereas others were hyporesponders. These differences were genetically determined. This may explain the heightened response in one of our five animals fed cholesterol. This animal developed cirrhosis.

The present findings indicate that the rhesus monkey can develop choline-deficiency cirrhosis, although it is less susceptible than the rat. The addition of cholesterol to the diet appears to exert an hepatotoxic effect.

Summary. One of five rhesus monkeys fed a diet deficient in choline and protein for 31 mo developed signs of cirrhosis at 26 mo. Five other monkeys were fed the same diet

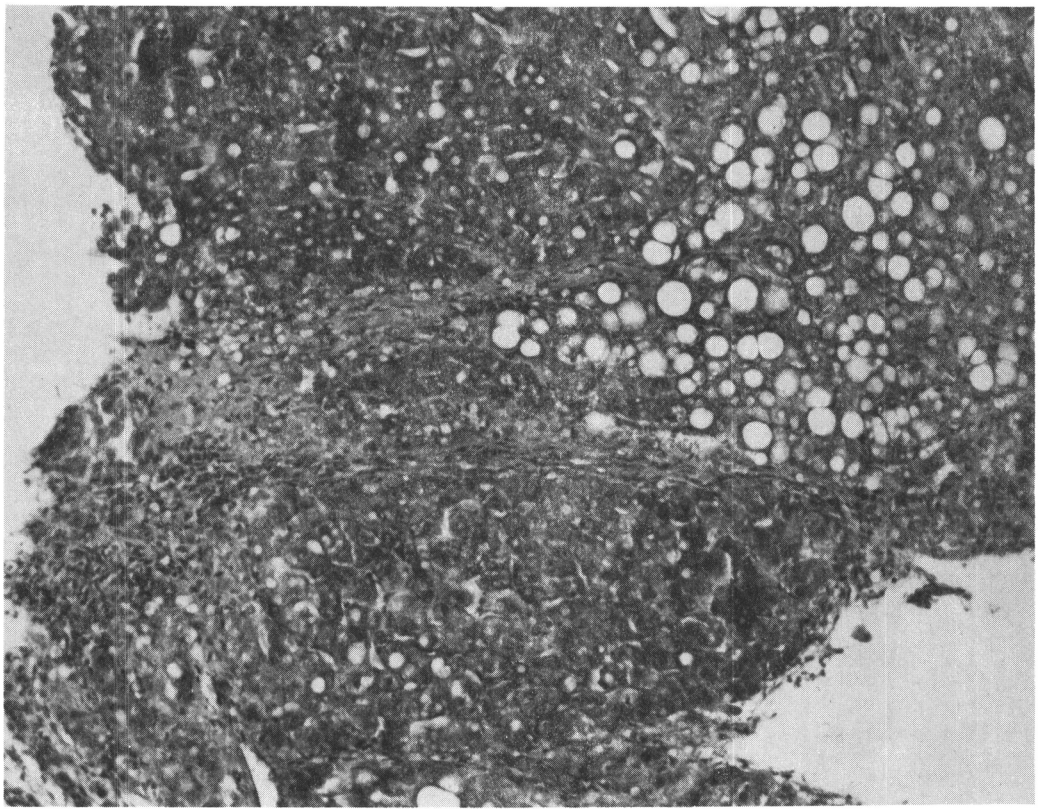

FIG. 3. Liver section from monkey in group Ib (cholesterol supplementation) showing broad scars, fatty changes and cellular disarray. Masson Trichrome ×100.

for 14 mo, at which time cholesterol comprising 2% of the diet was added. There followed a sharp rise in hepatic lipids. One monkey developed marked hypercholesterolemia and showed signs of cirrhosis 2 mo after cholesterol supplementation.

The findings indicate that the rhesus monkey is susceptible to choline-deficiency cirrhosis. They suggest that cholesterol supplementation accelerates this process.

This study was conducted at the New England Regional Primate Research Center, and supported in part under a grant from the Division of Research Resources, National Institutes of Health.

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Received February 25, 1974. P.S.E.B.M. 1975, Vol. 148