dium every 24 hours because of the high rates of metabolism and production of CO_2. Subculture is performed weekly by microsurgical dissection as the cells grow in tissue organoid form not unlike normal cytotrophoblast; in culture they reach a multilayer thickness of six to seven cells within 1 week. Dispersion with trypsin and other proteolytic agents has been possible, but subsequent viability is poor.

The BeWo line has maintained the cellular characteristics of the cytotrophoblast of the patient's original tumor, with no differentiation to syncytium in the rapidly growing log phase of growth. The line is highly aneuploid, varying widely in chromosome numbers from hypertriploid to octoploid.

Variations in the level of glucose in the medium exert profound changes on cellular growth and on glycogen content as shown by periodic acid–Schiff staining; the intensity of glycogen staining shown by this technique is very high in cultures maintained with glucose at 340 mg percent in the medium, whereas much less glycogen and less-vigorous growth are seen in cultures maintained on glucose at 140 mg percent. This decrease in growth rate can be seen in the increased time required by cultures on lower concentrations of glucose to reach the approximately 8-mm-diameter colony size when subculture is performed: with glucose at 340 mg percent in the medium, eight subcultures are possible within 60 days; with glucose at 140 mg percent, only four subcultures are possible. The utilization of glucose, the synthesis and breakdown of glycogen, and the participation of the key enzymes of the glycolytic cycle remain to be evaluated.

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Ethanol Increases Hepatic Smooth Endoplasmic Reticulum and Drug-Metabolizing Enzymes

Abstract. *Rats were fed ethanol for 2 weeks along with diets either adequate or deficient in protein and choline, the latter intake being similar to that of many alcoholics. Hepatic lipids, smooth endoplasmic reticulum, and the activities of drug-metabolizing enzymes (aniline hydroxylase and nitroreductase) were increased with the adequate diet but more so with the deficient one. These results may explain the increased tolerance by alcoholics of drugs such as sedatives.*

The association between drug-induced hypertrophy of smooth-surfaced endoplasmic reticulum (SER) in hepatocytes and increased activities of hepatic drug-metabolizing enzymes is well documented (*1*). Both in the rat (*2*) and in man (*3*) chronic administration of ethanol also leads to increase in and vesiculation of SER in hepatocytes. Because of these morphologic alterations and the possible clinical implications, it was of interest to determine whether chronic administration of ethanol affects hepatic microsomal drug-metabolizing enzymes.

Thirty-two male Sprague-Dawley rats, initially weighing about 250 g, were studied. One ("nondeficient") group of 12 animals was fed a described (*4*) complete diet in liquid form, with 18 percent of total calories as protein; the remaining 20 (the "deficient" group) received a deficient diet.

The two diets were identical except that the deficient diet lacked choline and 75 percent of casein, methionine, and cystine; these shortages were isocalorically replaced by carbohydrate. Ethanol was isocalorically substituted for carbohydrate and comprised 36 percent of total calories (*2*). Each rat fed ethanol was matched with a control rat that received the appropriate diet without ethanol.

After 15 days the animals were killed by exsanguination, and portions of the livers were homogenized in 0.25*M* sucrose at 0°C. In four pairs of animals fed the deficient diet, the microsomal fraction was isolated by centrifugation, total microsomal protein was measured, and the enzyme activities in washed microsomes were determined. The concentration of cytochrome P450 in isolated microsomes was measured in one pair from each group (*5*).

Contents of DNA, total lipids, and triglycerides (*4*) also were determined. Small blocks of liver were fixed in ice-cold 1-percent buffered osmic acid and embedded in Epon; ultrathin sections were then prepared for examination with the electron microscope.

The mean concentration of triglycerides in control animals was 2.5 times greater in the deficient group than in the nondeficient group ($P < .05$) (Table 1). After administration of ethanol, mean hepatic triglycerides increased fourfold in both groups ($P < .001$). The mean increase in hepatic triglycerides induced by ethanol was 2.4 times greater in the deficient group than in the other. Because of the greater content of hepatic lipids in the deficient animals, enzyme activities were expressed in terms of fat-free wet weight of the liver.

After administration of ethanol the mean aniline hydroxylase activity was strikingly increased in both groups; that of nitroreductase was moderately increased (Table 1). When the difference in aniline hydroxylase activity between control and alcohol-treated animals was calculated for individual pairs, the increase after administration of alcohol averaged 658 ± 140 units ($P < .001$) in the nondeficient and 1231 ± 181 units ($P < .001$) in the deficient group (means $\pm$ S.E.). The mean increase in aniline hydroxylase activity after administration of alcohol in the deficient group was therefore double that in the other group. Aniline hydroxylase activi-

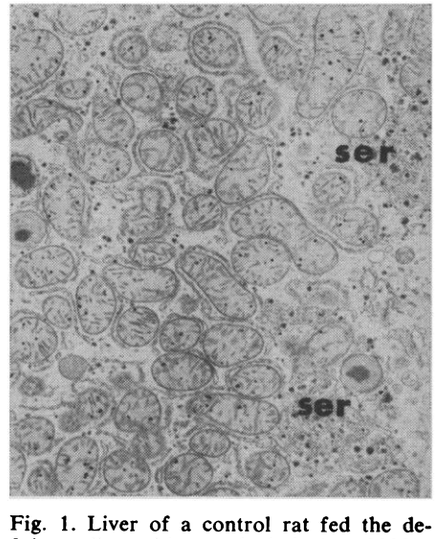

Fig. 1. Liver of a control rat fed the deficient diet without alcohol (× 9000). The smooth endoplasmic reticulum (*ser*) is not increased; mitochondria appear normal.

Table 1. Mean hepatic activities of aniline hydroxylase and nitroreductase and lipid content in rats given adequate or deficient diets, with or without alcohol. Activity of aniline hydroxylase determined from liver homogenates according to Imai and Sato (*12*). Enzyme units are millimicromoles of *p*-aminophenol formed per gram wet weight of fat-free liver in 15 minutes. Activity of nitroreductase determined from liver homogenates according to Fouts and Brodie (*13*). Enzyme units are micromoles of *p*-aminobenzoic acid formed per gram wet weight of fat-free liver in 15 minutes. Means with S.E.; A, alcohol.

Diet	Activities		Contents (mg/g, wet wt)	
	Aniline hydroxlyase	Nitroreductase	Total lipids	Triglycerides
Adequate	97 ± 13	1.19 ± 0.31	45 ± 3	11 ± 2
	$P<.001$	$P=.1$	$P<.001$	$P<.001$
Adequate +A	755 ± 141	1.71 ± .51	88 ± 8	44 ± 6
Deficient	127 ± 21	1.60 ± .09	67 ± 8	28 ± 8
	$P<.001$	$P<.001$	$P<.001$	$P<.001$
Deficient +A	1358 ± 163	1.89 ± .45	156 ± 17	108 ± 17

ties in control animals of both groups were essentially the same.

The mean increase in nitroreductase activity (for individual pairs) induced by ethanol was 0.83 ± 0.13 unit ($P < .001$) in the deficient group and 0.52 ± 0.26 unit ($P = .1$) in the other group. There were no significant differences in nitroreductase activity between alcohol-treated animals given the adequate diet and those given the deficient diet; similarly, no significant differences in nitroreductase activity were observed between control animals of both groups.

The data obtained from homogenates were confirmed by use of washed microsomes from four pairs of the deficient group. After administration of ethanol the mean activity of aniline hydroxylase in washed microsomes was increased sevenfold; that of nitroreductase was doubled ($P < .01$). When DNA content was used as a reference point for activities of aniline hydroxylase and nitroreductase, the results were comparable to those obtained by expression of activity in terms of fat-free wet weight.

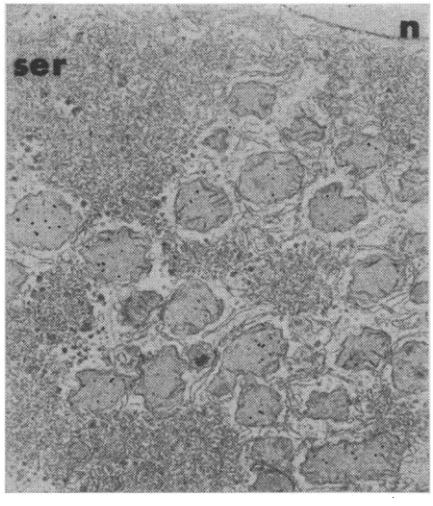

Fig. 2. Liver of a rat fed alcohol with the deficient diet (× 9000). The smooth endoplasmic reticulum is hypertrophied; many mitochondria are misshapen. *n*, Nucleus; *ser*, smooth endoplasmic reticulum.

Microsomal protein (determined only in animals on a deficient diet) increased from a control value of 25.3 ± 2.0 to 31.6 ± 0.9 mg per gram of fat-free wet weight after ethanol ($P < .05$). Feeding of ethanol also enhanced the concentration of microsomal cytochrome P450 (39 percent in the pair given the nondeficient diet and 80 percent in the pair fed the deficient diet).

Electron microscopically the control animals of both groups appeared normal (Fig. 1) except for slightly increased cytoplasmic lipid droplets in the deficient group. After administration of alcohol, alterations of mitochondria were observed similar to those reported (*2*). The SER was moderately hypertrophied in the nondeficient group and strikingly increased in the deficient group (Fig. 2).

The SER is considered to play an important role in the metabolism of drugs, in many instances by converting lipid-soluble materials to a water-soluble form for excretion (*6*). Administration of various pharmacologic agents, such as phenobarbital and diphenylhydantoin (*1*), induces an increase in SER and in the activities of hepatic drug-metabolizing enzymes. Ethanol is water-soluble, thought to be oxidized by alcohol dehydrogenase of the soluble fraction, and is almost completely oxidized rather than excreted. Thus it is surprising that chronic administration of ethanol leads to hypertrophy of the SER and increased activities of hepatic drug-metabolizing enzymes.

The increase in SER may not be a direct effect of alcohol but conceivably a response to the accumulation of lipids, which are in part processed in the SER, converted to water-soluble lipoproteins, and excreted into the blood. The fact that on a deficient diet both lipids and the activities of drug-metabolizing enzymes are increased more than on a complete diet supports this concept. The possibility, however, that alcohol itself is responsible, at least partly, for the hypertrophy of SER cannot be dismissed, since oxidation of alcohol by microsomes has been demonstrated (*7*).

The ethanol-induced increase in activity of aniline hydroxylase is much greater than that of nitroreductase. This finding is perhaps related to the fact that, whereas nitroreductase activity is equally distributed between rough endoplasmic reticulum and SER, the greater part of aniline hydroxylase activity is confined to the SER (*8*), which appears to be selectively increased by administration of ethanol (*2*, *3*).

The increase in activities of drug-metabolizing enzymes is not the only effect induced in the SER by ethanol; administration of ethanol also leads to increase in synthesis of cholesterol (*9*) and in circulating lipoproteins (*10*), both of which are assumed to reflect increased function of endoplasmic reticulum. The unusual tolerance by many alcoholics of such drugs as sedatives (*11*) may be related to increased levels of hepatic drug-metabolizing enzymes.

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