Table 2. Active sodium transport and adenosine triphosphatase activities in erythrocytes of parents of patients with cystic fibrosis compared with simultaneous controls. Pump II was significantly different at $P < .03$ by the rank-sum test of Wilcoxon.

Pump I	Pump II	Ouabain-sensitive ATPase
.75	.18	.92
1.19	.55	.72
1.18	.57	1.89
.96	.18	1.55
.73	.70	.80
$\bar{x} = .96 \pm .10$	$\bar{x} = .44 \pm .11$	$\bar{x} = 1.18 \pm .23$

controls were selected specifically in terms of age and sex although there is no evidence that the individual's age and sex is a determinant of the characteristics of the erythrocyte sodium efflux. The methods and calculations used have been described (*7*). The definitions of pumps I and II are as defined by Hoffman (*8*) and are as follows: pump I is that component of sodium efflux which is inhibited by the addition of ouabain to a medium which contains sodium and potassium; pump II is another decrement in the efflux of sodium which is due to the addition of ethacrynic acid to the medium which contains maximally inhibitable concentrations of ouabain. The remnant of the efflux—that is, the component of the total efflux unaccounted for by pumps I and II—is referred to as the "leak." This presumably correlates with the permeability of the red-cell membrane. The average value for sodium efflux in the controls for pump I was 2.47 mmole per liter of red cells per hour, and for pump II it was 0.45 mmole of sodium per liter of red-blood cells per hour. These data are comparable with our previously determined values among normals.

Ten patients with cystic fibrosis were studied with simultaneous control subjects; the mean decrease in pump I is 15 percent of the control value. However, pump II was more strikingly decreased in these patients. They were only 53 percent as active as their controls. These differences were statistically significant (Table 1). Furthermore, the "leak" parameter was statistically significantly less in the patients as well.

The proximate source of energy for the major component of sodium transport in the erythrocyte (pump I) is adenosine triphosphate. In addition, the glycoside-sensitive adenosine triphosphatase of red-cell ghosts is intimately linked to the glycoside-sensitive sodium efflux. Ghosts or fragmented red-cell membranes were prepared as reported (*9*). There was a mean decrease of 32 percent in the ouabain-sensitive adenosine triphosphatase activity of the seven patients studied (Table 1). In contrast, there was no difference between patients and controls in the ouabain-insensitive component of ghost adenosine triphosphatase activity. The mean value of the ratio of patient to control was 1.02.

Patients with cystic fibrosis have a normal concentration of sodium in erythrocytes but a diminished active efflux of sodium. This suggests that the passive influx of sodium (the "leak" parameter) might possibly be diminished, and the "leak" parameter of sodium efflux measured with isotope is diminished (Table 1).

In erythrocytes from parents of these patients there was no difference in pump I nor in the associated ouabain-sensitive adenosine triphosphatase activity; but there was a 56-percent diminution in pump II (Table 2). The magnitude of the depression in the activity of pump II is similar to that seen in the patients.

In summary, this investigation has demonstrated a diminished active transport of sodium in the erythrocytes of patients with cystic fibrosis. Of special significance is the marked decrease in that which is defined as pump II in the parents. This quantitative marker of the heterozygous carrier of the disease may be useful in genetic studies and in genetic counseling.

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References and Notes

1. P. A. diSant'Agnese, R. C. Darling, G. A. Perera, E. Shea, *Pediatrics* **12**, 549 (1953).
2. J. A. Mangos and N. R. McSherry, *Science* **158**, 135 (1967).
3. P. A. diSant'Agnese and R. C. Talamo, *New Engl. J. Med.* **277**, 1287 (1967).
4. A. Spock, H. M. C. Heick, H. Cress, W. S. Logan, *Pediat. Res.* **1**, 173 (1967).
5. B. S. Danes and A. G. Bearn, *Lancet* **1968-I** 1061 (1968).
6. Standard error of the mean.
7. J. R. Sachs and L. G. Welt, *J. Clin. Invest.* **46**, 65 (1967).
8. J. F. Hoffman, *Amer. J. Med.* **41**, 666 (1966).
9. A. Czerwinski, H. J. Gitelman, L. G. Welt, *Amer. J. Physiol.* **213**, 786 (1967).
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Hepatic Microsomal Enzymes in Man and Rat: Induction and Inhibition by Ethanol

Abstract. *The feeding of ethanol increased significantly the activities of hepatic pentobarbital and benzpyrene hydroxylases in rats, and, in human volunteers, doubled pentobarbital hydroxylase activity. In vitro ethanol inhibited aniline, pentobarbital, and benzpyrene hydroxylases. These data may explain, at least in part, the increased tolerance of alcoholics to sedatives when sober, and the enhanced sensitivity to sedatives when inebriated.*

We recently showed that ethanol ingestion in rats increases the activities of two hepatic, microsomal, drug-metabolizing enzymes, aniline hydroxylase and nitroreductase (*1*). Since the liver is not commonly called upon to metabolize aniline or nitrobenzoic acid, we proceeded to investigate the effect of chronic ethanol administration on the in vitro hepatic metabolism of a common sedative, pentobarbital, and a ubiquitous environmental carcinogen, benzpyrene.

Six female Sprague-Dawley rats, initially weighing about 200 g, were fed a liquid diet, deficient in protein and choline, as described previously (*1*). These animals were matched with six female littermates, which were pair-fed the same diet, in which ethanol, comprising 36 percent of total calories, was isocalorically substituted for carbohydrate. A deficient diet was employed, because when combined with ethanol, it led to greater increases in enzyme activity than the same dose of ethanol given with an adequate diet (*1*). After 14 days, the animals were killed by exsanguination and the hepatic activities of the following microsomal enzymes were determined in liver homogenates: pentobarbital hydroxylase and benzpyrene hydroxylase by the methods of Kuntzman *et al.* (*2*), and aniline hydroxylase according to Imai and Sato (*3*).

When compared with their pair-fed controls, the livers of rats treated with ethanol displayed increased activities of aniline hydroxylase, pentobarbital hydroxylase, and benzpyrene hydroxylase (Fig. 1). Thus, in rats, chronic intake of ethanol leads to increases in the activities of a wide variety of drug-metabolizing enzymes, including those involved in the detoxification of substances important to man.

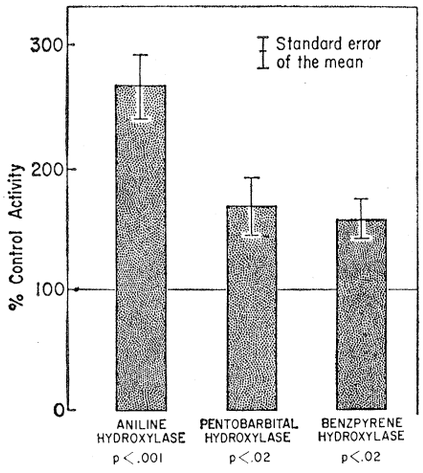

Fig. 1. Activities of hepatic aniline, pentobarbital, and benzpyrene hydroxylases in rats that had been fed ethanol (36 percent of total calories) for 14 days. Control levels, indicated as 100 percent, were as follows: aniline hydroxylase, 210 ± 25.4 nmole of *p*-aminophenol formed per gram of liver per 15 minutes; pentobarbital hydroxylase, 0.079 ± 0.009 nmole metabolized per milligram of liver per 10 minutes; and benzpyrene hydroxylase, 28.58 ± 2.43 ng of 8-hydroxy-benzpyrene formed per milligram of liver per 15 minutes.

To test directly whether ethanol increases hepatic, drug-metabolizing enzymes in man, three young nonalcoholic volunteers (two male, one female) were studied in the Clinical Research Center of the Mount Sinai Hospital. For 12 days they were fed an adequate diet containing 14 percent of total calories as protein, 33 percent as fat, 11 percent as carbohydrate, and 42 percent as ethanol. The diet was supplemented with additional vitamins and folic acid as described previously (*4*). No drugs or artificial sweetening agents were given during the experimental period. Aspiration liver biopsies were performed before administration of ethanol and at the end of the study. The control biopsy specimens were all morphologically normal. After administration of ethanol all biopsy specimens showed prominent fat accumulation. Pentobarbital hydroxylase activity was approximately doubled in each subject, while benzpyrene hydroxylase activity was essentially unchanged (Fig. 2). The changes in pentobarbital hydroxylase activity are comparable in man and rat, but the lack of increase in benzpyrene hydroxylase activity in the volunteers may indicate a species difference between man and rat.

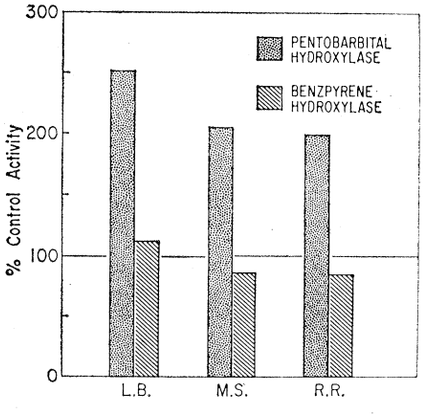

Fig. 2. Effect of 12 days of ethanol feeding (42 percent of total calories), in three nonalcoholic volunteers (L.B., M.S., and R.R.), on the activities of hepatic pentobarbital and benzpyrene hydroxylases. Control values, indicated as 100 percent, were as follows: pentobarbital hydroxylase, nanomoles metabolized per milligram of protein per 10 minutes, L.B. 0.081, M.S. 0.094, and R.R. 0.071; benzpyrene hydroxylase, nanograms of 8-hydroxybenzpyrene formed per milligram of protein per 15 minutes, L.B. 108, M.S. 86, and R.R. 74.

The induction by ethanol of enzymes that detoxify other drugs may explain the clinical observation that alcoholics, when sober, show increased resistance to the action of some sedatives (*5*); however, acute ethanol intoxication potentiates the action of barbiturates (*5*). We therefore investigated the effect of "physiologic" concentrations of ethanol on the in vitro hydroxylation of aniline, pentobarbital, and benzpyrene, measured by the procedures described, and on the reduction of nitrobenzoic acid, assessed by the method of Fouts and Brodie (*6*). Homogenates of rat liver were incubated with these substrates and 50 m*M* ethanol. As seen in Fig. 3, the activities of aniline, pentobarbital, and benzpyrene hydroxylases were inhibited by ethanol in a concentration commonly found in the blood of inebriated persons. By contrast, the activity of nitroreductase, an anaerobic system, was unaffected.

It is now apparent that ethanol and other drugs, exemplified by phenobarbital, share many characteristics. They both lead to hypertrophy of hepatic smooth endoplasmic reticulum and induce a nonspecific increase in a wide variety of hepatic, drug-metabolizing enzymes and in microsomal cytochrome P450 (*1, 7*). They increase hepatic cholesterol biosynthesis (*8, 9*). Both are oxidized by an aerobic, microsomal system that is dependent on the reduced form of nicotinamide adenine dinucleotide phosphate, and their metabolism is inhibited by carbon monoxide (*7, 10*). They inhibit the metabolism of other drugs in vitro (*7*).

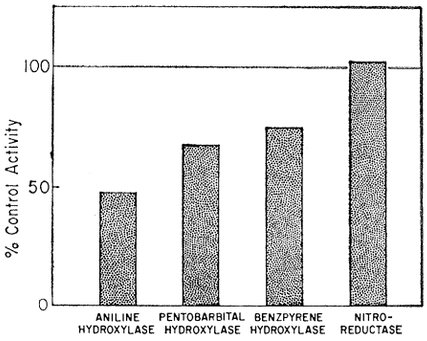

Fig. 3. In vitro effect of 50 m*M* ethanol on the activities of aniline hydroxylase, pentobarbital hydroxylase, benzpyrene hydroxylase, and nitroreductase in rat liver homogenates.

In conclusion, both in rat and in man, ethanol feeding increases the activity of hepatic, microsomal, drug-metabolizing enzymes. In vitro ethanol also inhibits the activities of these detoxifying systems. This suggests that in addition to possible changes in the central nervous system, the resistance of chronic alcoholics to drugs such as sedatives may be due, at least in part, to an ethanol-induced increase in hepatic drug-metabolizing activity, and that the inhibition of drug-metabolizing enzymes by ethanol may contribute to the increased sensitivity of inebriated persons to barbiturates.

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References and Notes

1. E. Rubin, F. Hutterer, C. S. Lieber, *Science* **159**, 1469 (1968).
2. R. Kuntzman, L. C. Mark, L. Brand, M. Jacobson, W. Levin, A. H. Conney, *J. Pharmacol. Exp. Therap.* **152**, 151 (1966).
3. Y. Imai and R. Sato, *Biochem. Biophys. Res. Commun.* **25**, 80 (1966).
4. E. Rubin and C. S. Lieber, *New Engl. J. Med.* **278**, 869 (1968).
5. K. Soehring and R. Schuppel, *Deutsche Med. Wochschr.* **91**, 1892 (1966).
6. J. R. Fouts and B. B. Brodie, *J. Pharmacol. Exp. Therap.* **119**, 197 (1957).
7. A. H. Conney, *Pharmacol. Rev.* **19**, 317 (1967).
8. C. S. Lieber and L. M. DeCarli, *Clin. Res.* **12**, 274 (1964).
9. A. L. Jones and D. T. Armstrong, *Proc. Soc. Exp. Biol. Med.* **119**, 1136 (1965).
10. C. S. Lieber and L. M. DeCarli, *Science*, in press.
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