dence, that association with cell lysosomes, followed by interaction with lysosomal membranes to produce permeability changes, are factors involved in the initial steps of the effects of these compounds on growth of intracellular tubercle bacilli, even though the nature of the interaction may well differ as between the surface-active ethers and the more inert polyvinylpyrrolidone, the charged suramin and uncharged dextran, and so forth. In this connection we have the difference in action on membranes by the long- and short-chained ethers, although so far shown directly only in nonlysosomal systems and after contact in vitro. A possible clue to the mechanism of their contrasting effects on tuberculous infection is offered by the histochemical evidence of differing lysosomal enzyme activity in infected surfactant-treated macrophages. The interpretation of this difference must be speculative at this stage, the possibilities being as diverse as (i) that reduced release of enzyme from lysosomes containing Macrocyclon leaves the acidic environment in the phagocytic vacuoles free to inhibit bacillary growth nonspecifically (*22*), whereas increased release from lysosomes containing HOC-60 produces a situation protective to the bacilli; or (ii) that surfactant-containing lysosomes selectively release into the cytoplasm inhibitory or growth-promoting substances affecting tubercle bacilli elsewhere in the cell (*11*); but a simpler possibility is not excluded, namely (iii) that the agents alter the surface of the bacterium, which is thus made less or more susceptible to attack by cell enzymes (*23*).

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23 August 1968

Red-Cell Transport Defect in Patients with Cystic Fibrosis and in Their Parents

Abstract. *The ouabain-sensitive and the ethacrynic acid–sensitive sodium efflux from erythrocytes of patients with cystic fibrosis are both decreased. Furthermore, the ouabain-sensitive adenosine triphosphatase activity is diminished in the red blood cell ghosts of these patients. Perhaps of greater significance is the fact that ethacrynic acid–sensitive sodium efflux is clearly diminished in the erythrocytes of the asymptomatic parents of these sick children. This defect in sodium transport may be valuable for detecting the heterozygous carrier state.*

There is an elevated concentration of sodium and chloride in the sweat of patients with cystic fibrosis (*1*). Mangos and McSherry (*2*) demonstrated that the sweat from patients with this disease inhibits sodium transport in the duct system of the rat parotid gland. The measurement of ion concentrations in sweat as a marker for the heterozygote state has been reviewed (*3*). Spock *et al.* (*4*) demonstrated that serum from patients with cystic fibrosis and their parents promoted a disorganization of the ciliary rhythm in explants of respiratory epithelium. Danes and Bearn (*5*) reported cytoplasmic intravesicular metachromasia with specific characteristics in skin fibroblast cultures from seven children with the disease and in 13 of the 14 parents. The study of Mangos and McSherry (*2*) motivated us to examine the components of active transport in another cell membrane system, the erythrocytes of patients with cystic fibrosis and their parents.

The mean concentration of sodium per liter of red blood cells in 18 children with the disease was 7.3 ± 0.39 mmole/liter (*6*). This compares well with the mean value in a group of 108 Caucasian controls in our laboratory, with a value of 7.2 ± 0.12 mmole/liter.

Sodium efflux studies were done with erythrocytes from patients and parents of patients, and each study was coupled with a control. In some instances the

Table 1. Active sodium transport and adenosine triphosphatase (ATPase) activity in erythrocytes of patients with cystic fibrosis. The ratio of activities of patients to controls of pumps I and II were significantly different at a $P < .005$; the adenosine triphosphatase activity at a $P < .023$, and the "leak" at a $P < .01$ as calculated by the rank-sum test of Wilcoxon. The definitions of pump I and pump II are those of Hoffman (*8*). $\bar{x}$, mean ± S.E.M.

Pump I	Pump II	"Leak"	Ouabain-sensitive-ATPase
.89	.15	.54	.59
.87	.66	.87	.45
1.02	.63	.83	.34
.66	.31	.71	.92
.92	.64	.59	.64
.88	.41	1.35	1.11
.88	.56	.40	.73
.69	.60	.91	
.89	.50	.52	
.77	.28	.67	
$\bar{x}$ =	$\bar{x}$ =	$\bar{x}$ =	$\bar{x}$ =
.85 ± .03	.47 ± .06	.74 ± .09	.68 ± .10

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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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.