Time Course of the Acute Alcoholic Fatty Liver and Concomitant Mitochondrial Function in Fasted Rats[1] (36907)

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A single moderate dose of alcohol[2] evokes a transient accumulation of hepatic triglycerides and an acute fatty liver in man and in laboratory animals (1, 2). Pyrazole, a potent inhibitor of liver alcohol dehydrogenase *in vitro* (3), effectively blocks ethanol metabolism and elimination *in vivo* (4–6). The ethanol induced hepatic triglyceride accumulation (after 20 and 44 hr) in male rats was inhibited by a 4 hr pretreatment with pyrazole (7). However, a 10 min pretreatment with pyrazole following a 7 hr fast did not prevent the fat accumulation in livers of female rats 16 hr after ethanol (8). Although the two experimental protocols varied considerably, these differing results were attributed to sex differences (9). Acute (10), but not chronic (11), treatment with ethanol enhanced materially hepatic mitochondrial respiratory control. Availability of an inhibitor of the *in vivo* metabolism of ethanol makes it possible to ascertain whether this enhancement of mitochondrial function resulted from a direct action of the compound or from the ensuing derangement of hepatic metabolism attendant to the oxidation of ethanol. Likewise it should be possible to determine if a similar mechanism accounts for the acute fatty liver in the same animals. Specifically, could enhanced mitochondrial function and ATP production stimulate endergonic lipid biosynthesis in the tissue? A time course study was designed to investigate these two phenomena mediated by ethanol. Animals were fasted during the experiment to obviate any effects due to unequal food consumption, and hence variable nutritional status, between groups.

Materials and Methods. Male albino rats (200–250 g, Holtzman Co.), maintained on Purina laboratory chow and water *ad libitum*, were divided into 4 groups: group C, control; group P, pyrazole; group A, alcohol; and group AP, pyrazole plus alcohol. The drinking water for groups A and AP was changed to 5% ethanol (v/v) 10 days before the start of experiments (10) whereupon these animals were injected intraperitoneally with 3 ml of 10% ethanol in saline/100 g body weight (3 g/kg). Ten minutes previously, group AP received 45 mg of pyrazole (0.66 mmole) in saline/100 g body weight by intraperitoneal injection. Group P received pyrazole only and group C received 3 ml saline/100 g body weight. All 4 groups were fasted for 24 hr before injections and were offered only drinking fluid thereafter. Five rats from each group were sacrificed by decapitation at 1, 4, 16, 24 and 39 hr after injection. Blood was collected with oxalate as anticoagulant and liver samples, freed of connective tissue, were taken for triglyceride analysis and for preparation of mitochondria. Blood levels of ethanol were determined enzymically with yeast alcohol dehydrogenase (EC 1.1.1.1) (12). The assay is valid since pyrazole inhibits the liver enzyme but not that of yeast (13).

Hepatic triglycerides were estimated by the method of Van Handel and Zilversmit (14) as modified by Forbes, Forbes and Patterson (15). Analyses were performed immediately on the fresh tissue since considerable losses occurred (probably by spontaneous hydrolysis) when tissues or homogenates were

1 Supported by the Bureau of Alcohol Studies and Rehabilitation, Department of Health, Commonwealth of Virginia.

2 The term alcohol is used synonymously for ethanol in this paper.

stored at —20°. Hepatic mitochondrial fractions were also prepared immediately (16) and respiratory control (control of mitochondrial respiration by phosphate acceptor) (17) was assayed polarographically (11) in duplicate. Mitochondrial protein was determined by a biuret method with deoxycholate (18).

Pyrazole (1,2-diazole) was obtained from Eastman Organic Chemicals and from J. T. Baker Chemical Co. and was recrystallized from petroleum ether prior to use. Biochemicals, including yeast alcohol dehydrogenase, were from Sigma Chemical Co.

Results. Rats lost about 29 g of body weight during the initial 24 hr fast with essentially no further loss thereafter.[3] Livers represented 3.3% of total body weight at sacrifice with no significant differences between groups or time periods. Following the initial 24 hr of fasting (zero time in Fig. 1) hepatic triglyceride content did not change for the remainder of the experiment in control (group C) animals and treatment with pyrazole alone (group P) had no effect. The rise in neutral fat within the first hour following the ethanol dose in groups A and AP was considered to result from fatty acid mobilization from adipose stores because of the rapidity of the response. In the former group triglyceride content decreased slowly over the subsequent 40 hr but in the latter a relatively rapid clearance of liver fat to control levels occurred within 24 hr despite the fact that prolonged, high blood alcohol levels were maintained in group AP rats (Fig. 1). Therefore maintenance of abnormal triglyceride concentrations cannot be accounted for by a direct action of ethanol *per se* because inhibition of its metabolism resulted in a return to normal triglyceride content even in the presence of high ethanol levels. Conversely, hepatic triglycerides did not decrease while

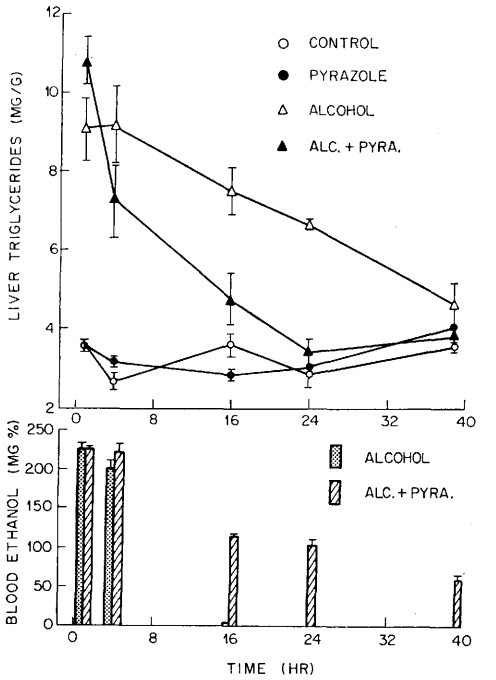

FIG. 1. Hepatic triglycerides and blood alcohol content in male rats following a 24 hr fast and treatment with ethanol and pyrazole, singly or in combination. Control, group C; Pyrazole, group P; Alcohol, group A; Alcohol + Pyrazole, group AP. Each point mean of 5 rats ± SEM.

the metabolism of ethanol was unhampered nor until *after* alcohol disappeared from the circulation. On the other hand, the approximately 3-fold rise in hepatic triglyceride in both group A and group AP during the first hour, while the blood alcohol curve was rising, implied that the very rapid response was unrelated to metabolism of ethanol or to *de novo* lipid synthesis, but rather to hormonally stimulated mobilization of fatty acids from fat depots (1).

It is well known that many metabolic dyscrasias arise from administration of ethanol; it enhances fat synthesis and depresses oxidation of fatty acids and triglycerides in the liver, stimulates mobilization of fatty acids from adipose tissue, and interferes with hepatic release of triglycerides (19, 1). Moreover, hepatic $NAD^+/NADH$ ratios were lower in rats following acute ethanol administration than in a similar group which received both pyrazole and ethanol (8, 9).

[3] No significant changes in body weights between groups occurred during the course of the experiment. Addition of 5% ethanol to the drinking water of groups A and AP 10 days prior to experimentation apparently did not alter their general nutritional status since mean body weights of group C, P, A and AP were 227, 228, 221 and 224 g, respectively, at the start of the fast.

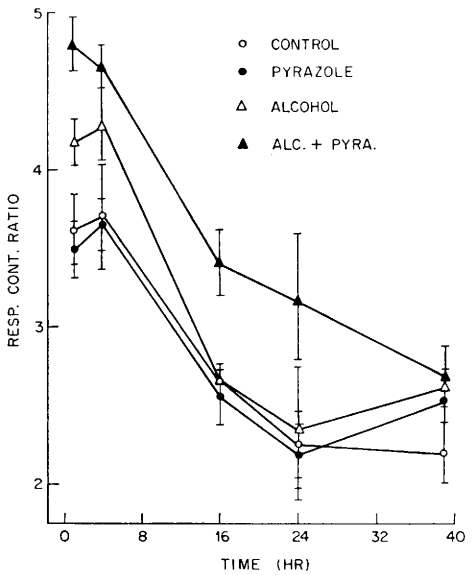

FIG. 2. Hepatic mitochondrial respiratory control. Same animals and groups as in Fig. 1. Calculated as the ratio of the respiratory rate (nanoatoms O_2/min/mg protein) in the active state (state 3) to that in the controlled state (state 4). Substrate, glutamate.

The fact that in the present study prolongation of the elevated triglyceride level was dependent upon the metabolism of ethanol, rather than to direct action of the alcohol *per se,* redirects attention to the role of an abundant supply of reducing equivalents as the stimulus for enhanced lipid synthesis in the genesis of the ethanol induced fatty liver (20).

Fasting has been shown to depress both the NAD^+/NADH ratio and the rate of ethanol oxidation in liver slices by about one-half. The first factor was considered to be the determinant of the second (21). Additionally, fasting mobilizes fatty acids from fat depots to the liver but it does not affect hepatic mitochondrial free fatty acids (10). Respiratory control in hepatic mitochondria also is depressed by a 24 hr fast and for at least an additional 24 hr (10). The latter effect was seen in the control animals in Fig. 2 and in the 3 experimental groups. The usual acute stimulation by ethanol (10) was observed and the response paralleled blood alcohol content. When ethanol metabolism was blocked with pyrazole enhancement of respiratory control was more pronounced and more prolonged. Pyrazole treatment alone did not affect respiratory control. Indeed, in both groups of alcohol treated animals (with or without pyrazole) the time course of the respiratory control ratios essentially coincided with that of the blood alcohol curve. These data verify that augmentation of respiratory control by ethanol resulted from the presence of ethanol *per se* and not from its metabolism. If the phenomenon were a consequence of ethanol metabolism, then pyrazole should depress and/or shorten its duration rather than enhance and sustain it. About 48 hr were required to clear blood of ethanol in the pyrazole treated group. In untreated fed rats the clearance rate was about twice that in these fasted animals. Essentially the same responses were obtained when glutamate was replaced by succinate as the mitochondrial substrate. In both cases, increases in the respiratory control ratios were due to stimulation of state 3 (ADP activated) respiration. These data, implicating a direct action of the ethanol molecule on mitochondria as an explanation for augmented respiratory control and efficiency of mitochondrial function, are in accord with previous proposals that ethanol increases the respiratory control ratio by promoting mitochondrial translocation of the adenine nucleotides ADP and ATP (10).

Discussion. The respiratory control ratio is considered the most important index of mitochondrial functional integrity and hence of the efficiency of ATP formation during substrate oxidation (22, 23). Although this capacity is increased by acute administration of ethanol (10), it probably has no putative role in the acute alcoholic fatty liver because triglyceride accumulation, but not augmented respiratory control, depended upon the subsequent metabolism of the alcohol. That is to say, liver fat dropped to the control level within 24 hr after blocking ethanol oxidation with pyrazole, whereas stimulated mitochondrial function disappeared within 16 hr *unless* ethanol clearance was blocked. The phenomena are best explained on the bases of marked depression of the NAD^+/NADH ratio during alcohol oxidation (thus favoring

the reductive synthesis of lipid) (20) and the attendant augmentation of respiratory control via enhancement by the ethanol molecule of mitochondrial translocation of the adenine nucleotides (10).

These relatively acute effects of ethanol administration to fasted rats differ from those observed in well nourished chronically treated animals. For example, when a nutritionally adequate liquid diet (40% of calories as ethanol) was fed for 4 wk, biogenesis of hepatic mitochondrial membranes was impaired resulting in damage to mitochondrial structure and function (24, 11). At the same time, liver triglycerides were not increased so long as the high lipotrope diet was fed (11). There is no question that disturbances in hepatic metabolism differ considerably in acute and chronic alcoholism.

Summary. Pyrazole, an inhibitor of liver alcohol dehydrogenase and ethanol metabolism, was used to distinguish between acute biochemical responses produced directly by alcohol and secondarily by its metabolic effects. Over the 40 hr period studied, accumulation of hepatic triglycerides was dependent upon an initial uptake of fatty acids from adipose stores and continued oxidation of ethanol by the liver. The increased efficiency of mitochondrial function, as monitored by the magnitude of respiratory control ratios, followed a time course isochronous with the blood alcohol curve and was dependent upon *in situ* exposure of the organelle to the alcohol molecule, not its metabolic sequelae. These relatively short-term responses are not sustained in well nourished animals receiving ethanol chronically.

The technical assistance of Mrs. Regina Foster is gratefully acknowledged.

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Received July 26, 1972. P.S.E.BM., 1972, Vol. 141.