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Presence of Nonoxidative Ethanol Metabolism in Human Organs Commonly Damaged by Ethanol Abuse

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Acetaldehyde, the end product of oxidative ethanol metabolism, contributes to alcohol-induced disease in the liver, but cannot account for damage in organs such as the pancreas, heart, or brain, where oxidative metabolism is minimal or absent; nor can it account for the varied patterns of organ damage found in chronic alcoholics. Thus other biochemical mediators may be important in the pathogenesis of alcohol-induced organ damage. Many human organs were found to metabolize ethanol through a recently described nonoxidative pathway to form fatty acid ethyl esters. Organs lacking oxidative alcohol metabolism yet frequently damaged by ethanol abuse have high fatty acid ethyl ester synthetic activities and show substantial transient accumulations of fatty acid ethyl esters. Thus nonoxidative ethanol metabolism in addition to the oxidative pathway may be important in the pathophysiology of ethanol-induced disease in humans.

THERE ARE OVER 10 MILLION ALCOholics in the United States, costing the economy $60 billion annually (*1*), but no generally accepted mechanism has been proposed that accounts for the propensity of certain individuals to drink to excess or to develop alcohol-related damage to organs. Recent studies demonstrated the existence of a heritable predisposition to abuse alcohol (*2, 3*). Because altered rates of acetaldehyde synthesis or degradation could explain this genetic component to alcohol dependency and organ damage, genetic analyses of the oxidative metabolism of ethanol to acetaldehyde (*4–6*) have been conducted.

It remains unclear why organs other than the liver develop alcohol-induced damage, especially the heart, pancreas, and brain, which lack or show minimal oxidative metabolism of ethanol and therefore are free of substantial acetaldehyde production (*7–9*). Moreover, selectivity of organ damage, such as the occurrence of alcohol-induced cardiomyopathy in the absence of liver or pancreatic disease (*10*), is difficult to understand because the extrahepatic organs damaged by ethanol are perfused with blood containing similar concentrations of acetaldehyde derived from the liver (*11*). Selective damage to organs that lack oxidative alcohol metabolism, therefore, suggests that mechanisms of alcohol-induced injury exist that are intrinsic to extrahepatic organs themselves. This is true especially since a genetic component for such organ injury has been observed in, for example, the brain (*2, 12, 13*). We report that ethanol is metabolized to fatty acid ethyl esters in many human organs. This finding suggests a role for nonoxidative ethanol metabolism in the development of damage to these organs.

For fatty acid ethyl ester isolation and gas chromatographic quantitation (*14*) in human organs, samples of heart (left ventricle), adipose tissue (abdominal panniculus), liver, pancreas, thoracic aorta, psoas muscle, cerebral cortex, cerebellum, and testes were obtained postmortem, with the addition of vitreous humor, kidney, and thyroid in some cases. A total of 175 organs were sampled from 20 randomly selected subjects.

The distribution of fatty acid ethyl esters reflected the type of ethanol exposure (Fig. 1). In acutely intoxicated subjects (n = 6), the concentrations of fatty acid ethyl esters were significantly higher than in controls in pancreas, liver, heart, and adipose tissue. Chronic alcoholics (n = 4) with undetectable blood alcohol concentrations had high fatty acid ethyl ester concentrations only in adipose tissue. Fatty acid ethyl esters were also detected in lower concentrations in some control subjects (n = 10), a finding probably due to social drinking within several days of death. Although high concentrations of fatty acid ethyl esters were present in adipose tissue in acutely and chronically intoxicated subjects, differences in the adipose content of the parenchymal organs, as reflected by fat vacuoles, were not responsible for the differences in the concentration of these esters between groups.

Of the parenchymal organs in acutely intoxicated subjects, fatty acid ethyl esters were most abundant in the pancreas, liver, heart, and brain (both cerebellum and cerebral cortex) (175, 121, 37, and 25 nmol/g, respectively), all target organs of ethanol-induced disease (Fig. 1). Lesser concentrations were found in the aorta, skeletal muscle, and testes (18, 13, and 15 nmol/g); the esters were absent in the thyroid, vitreous, and kidney. A typical gas chromatogram disclosed ethyl esters of palmitate, palmitoleate, stearate, oleate, linoleate, and arachidonate (Fig. 2). Moreover, in the acutely intoxicated and control groups, increasing blood alcohol concentration was linearly related to increasing fatty acid ethyl ester

Table 1. Fatty acid ethyl ester synthesis by human organs. Tissue was obtained at autopsy from control subjects, homogenized (10 percent, weight to volume) in 50 mM tris-HCl (pH 8), and incubated with [^{14}C]oleate (20,000 dpm/nmol; final concentration, 0.4 mM) and ethanol (final concentration, 200 mM) (*16*) for 60 minutes at 37°C. After addition of ethyl [^{3}H]oleate as a yield marker and ethyl oleate as a carrier, lipids were extracted with acetone and ethyl [^{14}C]oleate, the end product, was quantitated after thin-layer chromatography by scintillation spectrometry. Values are means ± standard errors.

Organ	n	Ethyl oleate produced (nmol g^{-1} hour^{-1}
Pancreas	5	468 ± 169
Liver	5	137 ± 28
Adipose	3	24 ± 9
Heart	6	11 ± 1
Brain (cerebral cortex)	4	11 ± 2
Skeletal muscle	3	7 ± 2
Aorta	3	7 ± 2
Buffer	5	<0.1

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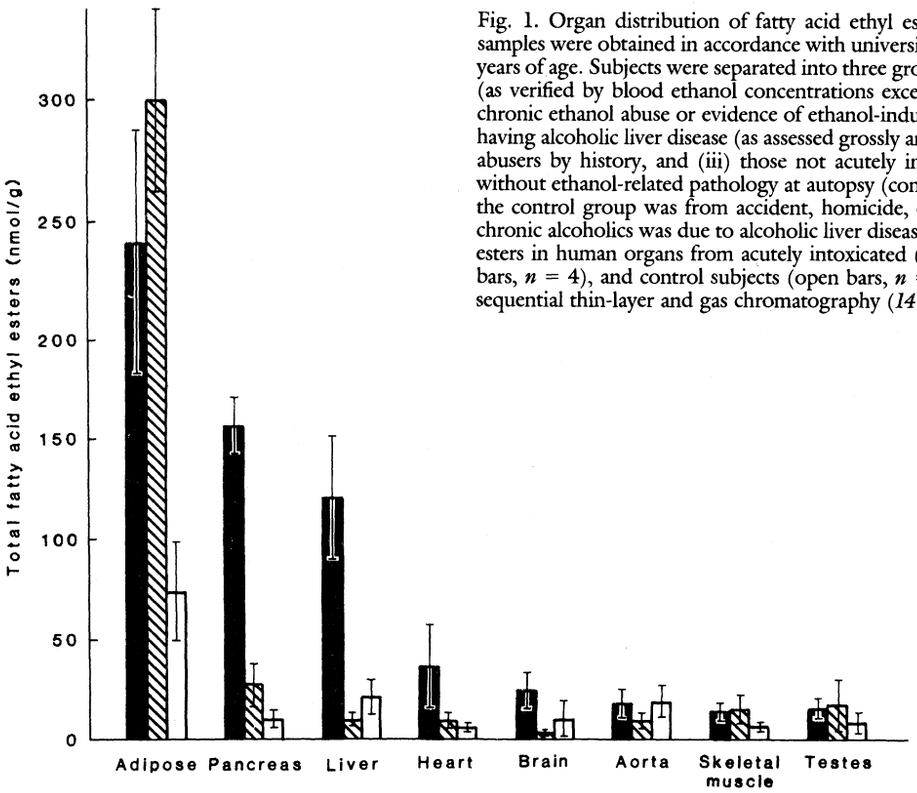

Fig. 1. Organ distribution of fatty acid ethyl esters in different alcohol exposure groups. Autopsy samples were obtained in accordance with university guidelines from males and females 6 months to 87 years of age. Subjects were separated into three groups: (i) those acutely intoxicated at the time of death (as verified by blood ethanol concentrations exceeding 80 mg per 100 ml) but without a history of chronic ethanol abuse or evidence of ethanol-induced pathology, (ii) those not acutely intoxicated but having alcoholic liver disease (as assessed grossly and microscopically) and known to be chronic ethanol abusers by history, and (iii) those not acutely intoxicated, without a history of ethanol abuse, and without ethanol-related pathology at autopsy (controls). Death in the acutely intoxicated group and in the control group was from accident, homicide, or non-alcohol-related natural disease. Death in the chronic alcoholics was due to alcoholic liver disease or accident. Concentrations of total fatty acid ethyl esters in human organs from acutely intoxicated (filled bars, n = 6), chronically intoxicated (hatched bars, n = 4), and control subjects (open bars, n = 10) were quantitated after acetone extraction and sequential thin-layer and gas chromatography (*14*). Results are expressed as means ± standard errors.

concentration in tissues (Fig. 3). A similar relation was noted in the liver and heart. Our findings suggest that fatty acid ethyl esters are formed during and shortly after ethanol ingestion in significant quantities in the pancreas, liver, heart, and brain and that they persist for prolonged periods in adipose tissue.

The capacity of normal human organs to synthesize fatty acid ethyl esters was assessed in the presence of added ethanol (Table 1). Fatty acid ethyl ester synthesis was observed in homogenates of pancreas and liver at 468 and 137 nmol g^{-1} (wet weight) $hour^{-1}$, respectively. Rates in heart, cerebral cortex, and adipose tissue were 11, 11, and 24 nmol g^{-1} $hour^{-1}$, respectively. Thus these organs contain an enzymatic activity (*15, 16*) responsible for catalyzing ethanol esterification, and the rates of synthesis in all organs except adipose tissue reflect the amount of accumulated fatty acid ethyl esters in acutely intoxicated subjects.

Our results show that fatty acid ethyl esters, the esterification products of ethanol with various fatty acids, are present in high concentrations and are synthesized at high rates in human organs frequently damaged by chronic ethanol abuse. After ethanol ingestion, the liver and pancreas manifest the highest fatty acid ethyl ester concentrations (Fig. 1) and the highest rates of synthesis (Table 1). Correspondingly, these two organs are the most commonly affected by ethanol-induced injury. The heart and brain, which are also damaged by chronic ethanol ingestion (although less frequently), display less but still significant fatty acid ethyl ester accumulation and synthetic activity. Organs not usually associated with ethanol-induced disease (thyroid and kidney) and organs only occasionally damaged (skeletal muscle and testes) accumulate minimal amounts of fatty acid ethyl esters and contain low synthetic activity.

Since heavy ethanol exposure alone is not always correlated with the presence or severity of ethanol-induced disease (*17*), most but not all (*18*) biochemical investigations have focused on genetic alterations of the enzymes of oxidative ethanol metabolism (*4–6*), either alcohol or acetaldehyde dehydrogenase, the only human proteins purified to homogeneity that directly interact with ethanol or its metabolites (*19, 20*). The underlying assumption is that genetic modulation of acetaldehyde concentrations determines susceptibility to alcohol dependency and alcohol-induced disease.

No unifying concept, however, has related oxidative metabolism of alcohol to the clinical and epidemiological data showing selective patterns of alcohol-induced damage. For instance, alcohol dehydrogenase, the enzyme that metabolizes most of ingested ethanol and which resides predominantly in the liver (*21*), exhibits very low activity in the pancreas and in most areas of the central nervous system and is absent from the heart (*8, 9*). Catalase, an alcohol-metabolizing system in the rat (*22*), has not been shown to oxidize ethanol in vivo in humans, and

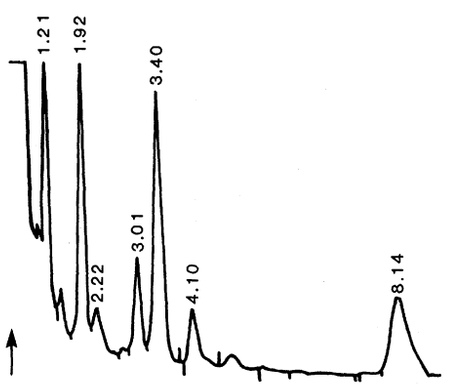

Fig. 2. Gas chromatogram of fatty acid ethyl esters from the left ventricle of a subject acutely intoxicated at the time of death. Retention times of 1.92, 2.22, 3.01, 3.40, 4.10, and 8.14 minutes serve to identify ethyl esters of palmitate, palmitoleate, stearate, oleate, linoleate, and arachidonate, respectively (*14*). An internal standard, methyl myristate, elutes at 1.21 minutes.

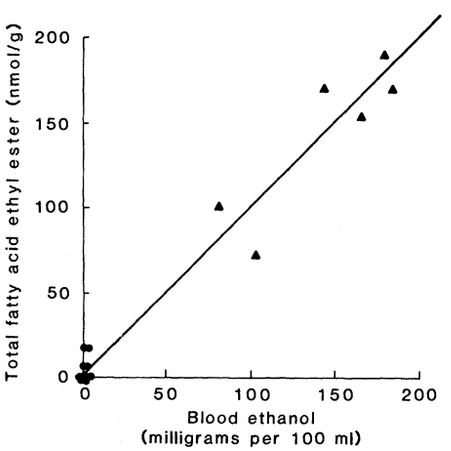

Fig. 3. Relation of blood ethanol concentration to pancreatic fatty acid ethyl esters concentration. Ethyl esters were isolated and quantitated from 1 to 2 g of pancreas from subjects acutely intoxicated at the time of death (n = 6) and from controls with no blood ethanol detectable (n = 10). Slope ± standard deviation, 0.95 ± 0.12.

some organs such as the heart lack the microsomal ethanol-oxidizing system (*7*) yet are targets of alcohol-induced damage. Although acetaldehyde is highly reactive and could damage hepatocytes, its concentration in blood may be markedly overestimated (*11, 23*). Indeed, liver-produced acetaldehyde has not been demonstrated to directly injure organs that lack endogenous production of this aldehyde. Finally, the genetic components of the selectivity of organ damage induced by ethanol (*2, 12, 13*) and the development of alcohol-induced heart muscle disease in the absence of pancreatitis or cirrhosis (*10*) cannot be attributed to acetaldehyde in blood because each of the organs is exposed to similar concentrations of circulating acetaldehyde derived from the liver (*11*). Thus there must be gene products residing in such organs that ultimately produce or modulate tissue injury and are responsible for the selectivity of organ damage.

These considerations suggest that other biochemical mediators are involved in alcohol-induced organ injury. Our results indicate that a nonoxidative pathway for ethanol metabolism exists in the human organs that are commonly injured by alcohol abuse but lack oxidative ethanol metabolism. Thus fatty acid ethyl esters could be such mediators involved in the production of alcohol-dependent syndromes. In organs that lack oxidative pathways, organ specificity of ethanol-induced injury may be related to rates of fatty acid ethyl ester synthesis and degradation. Of course, interaction between the oxidative and nonoxidative pathways may occur.

A potential mechanism of injury by fatty acid ethyl esters was recently demonstrated in isolated mitochondria (*24*). The fatty acid ethyl esters, acting as a shuttle for fatty acid between cellular fatty acid binding sites and the mitochondria, bind to these organelles where they are hydrolyzed and release free fatty acids, known uncouplers of oxidative phosphorylation. Impaired mitochondrial function has been well established in alcoholic cardiomyopathy, and the release of free fatty acids has been proposed as one of the initial events leading to alcohol-induced pancreatitis (*25*) as well as alcohol-induced heart disease (*26*). Since the amount of fatty acid ethyl ester formed in a parenchymal target organ (Fig. 3) is linearly related to blood alcohol concentrations, individuals who ingest large amounts of alcohol will synthesize more fatty acid ethyl esters.

In summary, nonoxidative ethanol metabolism occurs in humans in the organs most commonly injured by alcohol abuse. Since some of these organs lack oxidative ethanol metabolism and because acetaldehyde cannot be the sole chemical mediator producing selective damage, fatty acid ethyl esters and their metabolism may have a role in the production of alcohol-induced injury. Thus fatty acid ethyl esters as well as acetaldehyde or other as yet unidentified chemical agents may serve as a link between alcohol intake and development of alcohol-induced disease.

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Optical Image Quality and the Cone Mosaic

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Contrary to the orthodox view that optical image quality should "match" the photoreceptor grain, anatomical data from the eyes of various animals suggest that the image quality is significantly superior to the potential resolution of the cone mosaic in most retinal regions. A new theory is presented to explain the existence of this relation and to better appreciate eye design. It predicts that photoreceptors are potentially visible through the natural optics.

THE IDEA THAT THE PHOTORECEPTOR mosaic should "match" the optical image quality is well entrenched in the vison literature. It is implicitly assumed that the optical image quality is fixed and that a photoreceptor grain evolved to best encode it. Using comparative biological data, we now show that this classical belief is wrong. In fact, the data are consistent with the opposite view, that the retinal grain is set by biological needs and that it is the optics that molds itself to best serve the retina. This simple biological explanation has major new implications for understanding the design of photopic eyes, particularly those with highly differentiated retinal topography such as visual streaks. It predicts correctly that optical image quality should be significantly superior to the photoreceptor grain and, in animals with nonuniform retinas, be adaptively modulated away from the optic axis rather than passively deteriorating due to classical aberrations.

Few biological data are available, but it appears that cone undersampling is widespread, if not the general rule. Several examples are shown in Table 1, which gives the sampling bandwidth or highest spatial frequency ν_s that can be unambiguously reconstructed by the cone mosaic; the optical bandwidth or maximum spatial frequency ν_o passed by the optics in bright light (smallest pupil diameter); the sampling efficiency ν_s/ν_o, which specifies the amount of undersampling; the posterior nodal distance of the eye P; and the ratio d/d_c, where d is the cone diameter and d_c the distance between cone centers (*1*). Matched sampling occurs when the anatomical resolving power equals the optical resolving power ($\nu_s = \nu_o$), or, equivalently (*2*), when the half-height width $\Delta\rho$

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