0023-6837/84/5001-0005$02.00/0
LABORATORY INVESTIGATION

Vol. 50, No. 1, p. 5, 1984
Printed in U.S.A.

Biology of Disease

Alcoholism and Aldehydism: New Biomedical Concepts

JEAN-PIERRE VON WARTBURG AND ROLF BÜHLER

Medizinisch-chemisches Institut der Universität Bern, Bern, Switzerland

New results of biomedical research in alcoholism show great interindividual, as well as racial, variability with respect to metabolism of alcohol and its first oxidation product, acetaldehyde. Genetic factors play an important part. The enzymes of alcohol and aldehyde metabolism exhibit a genetically determined heterogeneity (isoenzymes and enzyme polymorphisms). This leads to a large variety of individually different enzyme phenotypes. Thus, the hypothesis is put forward that the individual and racial differences in alcohol metabolism are based on the genetically determined variability of the participating enzymes, alcohol dehydrogenase and aldehyde dehydrogenase.

Alcohol metabolism and alcohol-induced disturbances of the intermediary metabolism are closely interrelated. Hence, genetic codetermination can also be expected in this regard.

As a toxic intermediate of alcohol metabolism, acetaldehyde plays a central role. Three positive ranges of acetaldehyde levels can be defined: (a) the normal range, (b) the "acute aldehyde syndrome" with extremely high levels of acetaldehyde, (c) "chronic aldehydism" with slightly elevated acetaldehyde levels.

In Orientals lacking the mitochondrial low K_m aldehyde dehydrogenase, acetaldehyde accumulates and produces symptoms of intoxication. This acute aldehyde syndrome is highly aversive and, thus, prevents these individuals from drinking. The effect is similar to the consequences of the inhibition of aldehyde dehydrogenase with disulfiram, a commonly employed drug in the treatment of alcoholics.

In alcoholics slightly elevated levels of blood acetaldehyde are observed. There are indications that this chronic aldehydism is not only the consequence of excessive drinking but may reflect a preexisting enzyme pattern that is genetically determined. Therefore, slightly elevated acetaldehyde concentrations could serve as a biologic marker for high risk drinking.

Alcohol dehydrogenase is found in many organs and often in specialized cells within a particular tissue. A specific enzyme pattern in these cells could lead to elevated acetaldehyde concentrations, consequently inducing acetaldehyde-related damage. Such organs could represent direct targets even at low blood acetaldehyde levels.

Medical doctors are confronted daily with the consequences of an uneven distribution of alcohol consumption in our countries (Fig. 1): a small percentage of the population (5 to 10%) drink a third or even up to half of all the consumed alcohol, which in Western industrial countries leads to an average daily consumption of 80 to 120 gm of pure alcohol by these people. On the other hand it is a well-known fact that the majority of the people in an alcohol-consuming society show a lifelong pattern of little or moderate drinking without developing alcohol-related problems. Moreover, genetic studies reveal that not everybody is at equal risk to develop alcohol-related disorders. The importance of genetic factors in alcoholism is being stressed by family, twin, and adoption studies.

Early detection of alcoholics as well as prevention and treatment of alcoholism are hampered by poor knowledge of the biomedical and psychosocial factors that predispose to or protect from the development of alcoholism. Of particular interest are genetically determined factors that could increase the risk of specific target organs to sustain alcohol-induced damage, or genetic factors that could lead to a change in the individual drinking behavior via biochemical mechanisms.

Recent data confirm the fact that the risk of chronic physical damage increases continually with the amount of alcohol consumed and the period of drinking. Hence, no generally valid threshold value for a safe alcohol consumption can be given. On the contrary, great differences in the susceptibility of various target organs in individual consumers as well as different susceptibility between individuals exist. The average dose-effect curve is best established in liver cirrhosis: in male subjects the risk triples upon consumption of 21 to 40 gm of alcohol/day and increases 600-fold with a daily amount of 140 gm of alcohol (58, 59), but even with such large quantities

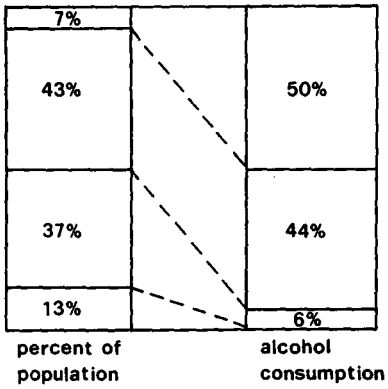

FIG. 1. Distribution of alcohol consumption.

only a small minority of individuals actually develop liver cirrhosis. In women as little as 20 gm/day can lead to alcoholic cirrhosis. Thus, there is a striking contrast between the relatively high susceptibility of occasional drinkers and the relatively remarkable resistance of some heavy drinkers. These differences in susceptibility might at least partly be due to genetic factors. However, no data are available with respect to the relative importance of genetic and environmental factors that determine the susceptibility of alcohol-induced organ or tissue damage. There is no doubt that both biologic and psychosocial factors play important parts and that the interplay between these factors has been underestimated. In this regard investigations of the alcohol metabolism, notably with respect to the metabolite acetaldehyde and the enzymes by which it is produced and removed and how these are influenced by genetic and environmental factors, have provided interesting indications.

FATE OF ETHANOL IN THE BODY

The processes that take place in the organism after ingestion of ethanol can be divided into three phases: the absorption phase, the distribution or diffusion phase, and the elimination phase (Fig. 2) (35, 83).

During the absorption phase ethanol is absorbed in the upper parts of the gastrointestinal tract purely by diffusion; there is no need of preceding digestion by hydrolytic enzymes as is the case with other food components. However, a partial oxidation of the alcohol by the alcohol dehydrogenase (ADH) in the gastrointestinal mucosa cannot be excluded (40, 84). The absorption rate depends on factors such as the amount of alcohol and concentration and composition (additives) of the alcoholic beverages, as well as on the food intake (dilution, time of passage through the various fast absorbing intestinal parts, blood supply of the mucosa). The average rate of increase of the blood alcohol concentration can vary from about 30 to 270 mg/100 ml in 1 hour (99). Peak alcohol levels occur between 20 and 120 minutes. The absorption phase is usually terminated after 2 hours and overlaps with the diffusion phase.

The most simple pharmacokinetic description of absorption is based on first order kinetics, whereby the rate of absorption at a given time is proportional to the quantity of alcohol still to be absorbed. As the rate of absorption is different for stomach and duodenum, the gastric emptying rate also plays an important part. Therefore, a correspondingly complicated absorption model contains two absorption constants (stomach and intestine) as well as a first order rate constant for gastric emptying (95). Since the absorption kinetics essentially determine the blood alcohol concentration with which the liver is perfused during the absorption phase, they play an eminent role in the elimination kinetics during this phase.

During the diffusion phase the alcohol is distributed in the tissues essentially in proportion to their water content (interstitial and intracellular water plus blood). On the average the total volume of body water is larger in men than in women; therefore, corresponding sex-related differences in blood alcohol concentrations occur even after ingestion of identical amounts. In addition, the total volume of body water is age dependent. During pregnancy alcohol is also distributed within a few minutes into the amniotic fluid and the fetal body water (88). During absorption the arterial blood levels are higher than the concentrations in venous blood because alcohol still diffuses from the intravasal water compartment into extravasal water. During elimination the transcapillary diffusion occurs in the opposite direction, and the venous concentrations are slightly lower. A mathematical model for the ethanol exchange process over the capillary membrane has recently been described (93).

Experiments with ^{14}C-labeled ethanol show that all of the perorally administered alcohol is absorbed and that only a few percent are excreted unmetabolized by the lungs, in urine and sweat (12, 82). Older work has also demonstrated that ethanol is fully absorbed with and without food intake (17, 28, 73).

The elimination phase is characterized by a decrease in blood alcohol concentration, whereby approximately 90% of the elimination is accomplished by oxidation of

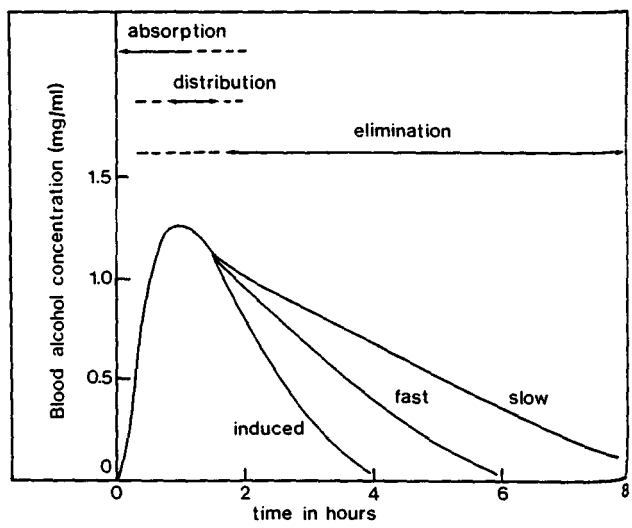

FIG. 2. Schematic representation of blood alcohol curves. Slow, fast and induced elimination rates result from corresponding ADH and MEOS patterns.

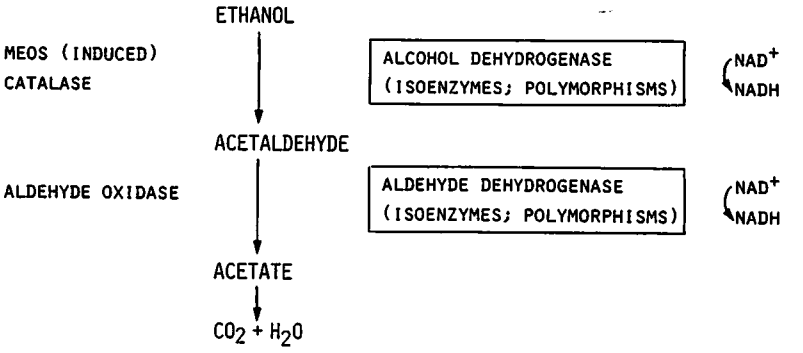

FIG. 3. Ethanol metabolism and its enzymes.

ethanol to carbon dioxide and water, thus representing the main pathway. In the early literature at the beginning of the century there was already a fierce controversy regarding the linearity of the decrease of the blood alcohol curve (for reviews see references 17, 95). At that time little was known about enzyme kinetics according to Michaelis and Menten so that an exponential decrease was assumed with a nonlinear course. The variation in the route of ethanol administration as well as species differences played an important part in the occurrence of so many diverging observations. Finally, however, the dogmatic opinion prevailed that the oxidation of alcohol was independent of the ingested quantity or the given blood alcohol concentration, respectively. These zero-order kinetics found expression in Widmark's mathematical formulations (94).

By means of numerous determinations using the Widmark method, an elimination rate of 100 mg/kg/hour was established, with a substantial scattering of 70 to 130 (17). At many places these empirical values are still fundamental in practical forensic medicine.

For quite some time observations in animals and humans gave rise to doubts about the exclusive validity of the dogma of dose and concentration independence of alcohol oxidation: repeatedly, dose-dependent kinetics or curvilinear shapes of blood alcohol curves have been described. An important step toward a new interpretation of blood alcohol curves was accomplished by Lundquist and Wolthers (48) in 1958 who essentially suggested a one-compartment open model with Michaelis-Menten elimination kinetics. During the 1970s it was mainly the extensive work done by Wagner and coworkers that led to refinements and extension of the models and finally to the development of pharmacokinetic notions which allowed a mathematical description of the entire blood alcohol curve during the resorption, diffusion, and elimination phases (79, 80, 95). With these models nonlinear least squares regression analysis of experimental blood alcohol curves and the estimation of elimination parameters such as the Michalis constant (K_m) and the maximal velocity (V_{max}) are possible. Furthermore, they allow one to carry out computer simulations in order to better understand the interferences of the various pharmacokinetic parameters such as absorption and elimination constants and the area under the curve. Yet, these models are still not able to provide an adequate description of blood alcohol curves observed in drinking experiments under a variety of circumstances. This is mainly due to the fact that they still neglect such phenomena as the first pass elimination by the liver or the multitude of enzymes involved in alcohol metabolism.

Ethanol Oxidation and Its Enzymes

Ethanol is almost exclusively eliminated from the body by oxidation. This process takes place primarily in the liver, whereby ethanol is metabolized enzymatically first to acetaldehyde and then to acetate (41, 44, 83). The enzymes which catalyze this two-step oxidation of ethanol to acetate are ADH and aldehyde dehydrogenase (ALDH) (Fig. 3). On the other hand, a small part of the ethanol, 10% or less, can be metabolized by alternative pathways, *i.e.*, the microsomal ethanol-oxidizing system (MEOS) and catalase (44). A mechanism involving hydroxyl radicals has recently been proposed (96). The activity of the MEOS can be induced by various drugs and by chronic alcohol intake. Very little of the consumed ethanol is excreted or exhaled when ethanol concentrations in blood are less than 200 mg/100 ml or 40 mM.

In the older, as well as in the more recent, literature great interindividual differences regarding alcohol elimination rates are reported, whether they be calculated by the now obsolete method of Widmark or by using Michaelis-Menten kinetics. They differ 2- to 3-fold after ingestion of identical amounts of alcohol (3, 17, 65, 79). Such large interindividual differences can be due to a combination of genetic and environmental factors. Newer data indicate that approximately half of the differences in the alcohol elimination rates observed between individuals are genetically determined, whereas the remainder is probably due to environmental factors (38). Several investigations dealing with alcohol metabolism in various racial groups also point to genetic influences. Differences were sought in the absorption, the elimination from the blood, and the oxidation rate between North American Indians, Chinese, Japanese, Europeans, and white Americans. In most studies a statistically significant increase in the alcohol degradation rate was found in Orientals and American Indians as compared with Caucasians (21, 24, 64, 65).

Thus, the question arises whether these individual as well as racial differences in alcohol metabolism are due to genetically determined differences in the respective enzyme patterns. Indeed, several research groups have observed that the activity of human liver ADH shows a considerable interindividual variability (43, 53, 68, 69, 91). Consequently, one can assume that the differences

in alcohol metabolism probably mainly reflect the broad range of the catalytic properties of human ADH, which in turn is due to the large number of multiple molecular forms observed in this enzyme (Table 1).

Many multiple forms can be ascribed to the presence of isoenzymes. They occur by random combination of three different subunits (α, β, γ) to the six possible dimeric forms (41, 68, 71, 72, 87). Further enzyme forms are due to enzyme polymorphisms. At the gene locus coding for γ-polypeptide chains there are allelic genes that lead to γ_1- and γ_2-subunits. Apparently, several polymorphisms occur at the gene locus that codes for the β-subunits: in addition to the normal β_1-subunit the "atypical" β_2-subunit (β-Bern) (91, 92) or a β-Indianapolis subunit (discovered there) can occur (5, 42). In addition to these forms other ADHs have been identified. π-ADH is present in all humans, although to a variable extent (43). Finally, forms with a cathodic electrophoretic mobility (χ-enzyme) were also described (57), the structure and gene control of which are still unknown.

The K_m for ethanol as substrate for the various forms of human liver ADH varies considerably. The main isoenzyme $\beta_1\beta_1$ isolated from normal liver homogenates reveals nonlinear kinetics with a lower K_m of 0.5 mM and a higher K_m of approximately 8 mM and equal contributions to the maximal velocity (6, 9, 15, 80). Hence, the higher value is similar to the one observed for the MEOS which has been found to be 8 to 10 mM (45). Finally, an even higher value of 15 to 30 mM has been reported for the π-enzyme (64, 65), and with the x-enzyme saturation with ethanol could not be reached (57). From these findings the question arises as to what extent the variable affinities of the multiple enzyme forms also affect alcohol elimination *in vivo*. During the phase of absorption from the gastrointestinal tract the relatively high alcohol concentrations in the portal vein should lead to increasing saturation of the high K_m forms of ADH, so that a considerable amount of alcohol could be removed by the first pass elimination during absorption (Fig. 4). Alcohol eliminated by such a mechanism would not appear in the peripheral blood, although it has been absorbed in the gastrointestinal tract. Consequently, an apparently incomplete absorption would result from this phenomenon and the area under the peripheral blood alcohol curve would be diminished in spite of an actually complete absorption.

Obviously, the availability of the coenzyme is a prerequisite for the effectiveness of high K_m forms of human liver ADH for a first pass elimination of alcohol during absorption. The activity of ADH in the liver of atypical individuals is considerably elevated. Hence, one might expect that such individuals could eliminate alcohol at a much faster rate than normal. However, a few atypical individuals were found to have only a slightly higher rate during the steady state of alcohol metabolism (16, 92). This fact indicates that in these individuals the reoxidation of NADH becomes the main factor limiting the rate of alcohol oxidation. However, during the initial phase of alcohol metabolism, *i.e.*, immediately after drinking an alcoholic beverage and during the absorption of the alcohol in the gastrointestinal tract, NADH reoxidation would be less limiting until the pool of oxidized NAD^+ is depleted and the reoxidation state (NADH to NAD^+) has reached equilibrium in the new, more reduced state in the whole organism. Furthermore, an adaptive increase in the rate of ethanol metabolism may occur rapidly after the ingestion of alcohol (swift increase in alcohol metabolism) (76). The phenomenon is based on

TABLE 1. POLYMORPHISM OF HUMAN LIVER ALCOHOL DEHYDROGENASE

Enzyme class	Gene locus	Alleles	Peptide chains	K_m ethanol
				mM
I	*ADH-1*		α	
	ADH-2	1	β_1	
		2	β_2 (β_{Berne}, "atypical")	<5
			$\beta_{Honolulu}$	
			$\beta_{Indianapolis}$	
	ADH-3	1	γ_1	
		2	γ_2	
II	?	?	π	34
III	?	?	χ	Very high

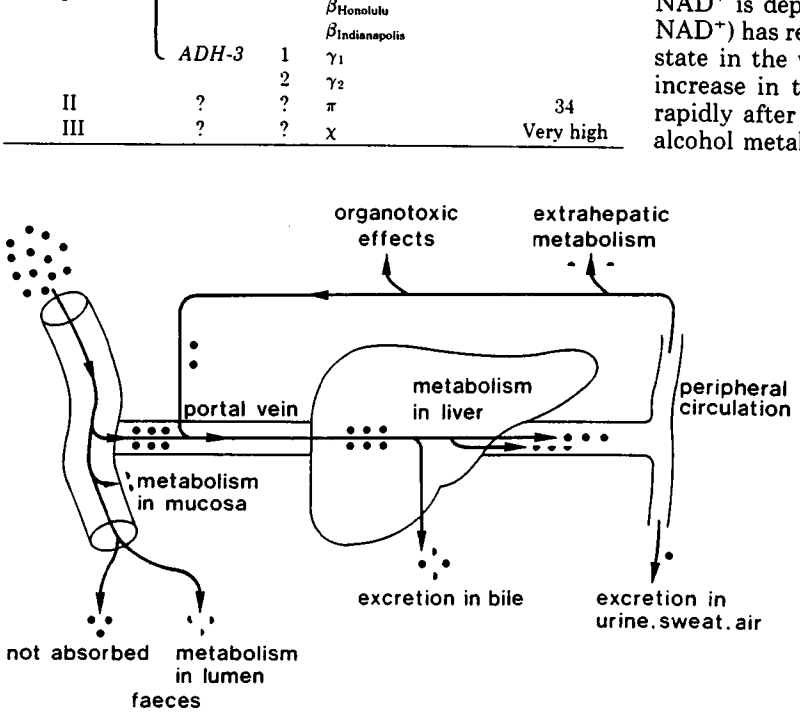

FIG. 4. Sites of ethanol removal with first pass oxidation in the liver during absorption.

an increase in the mitochondrial reoxidation of NADH, and catecholamines are probably involved in the mechanism (98). Swift increase in alcohol metabolism seems to occur in humans within one drinking episode but apparently not in all individuals (R. G. Thurman, personal communication). This observation is of great importance because swift increase in alcohol metabolism could allow for enhanced ethanol oxidation during and shortly after absorption in individuals with corresponding isoenzyme patterns of ADH (high V_{max} and high K_m).

The pharmacokinetics of ethanol which determine both the shape and the area under the blood alcohol curve thus depend in a complex way on the enzyme patterns (89). The length of time during which the alcohol remains in the organism, however, is of decisive importance for the immediate toxic effect. Hence, one has to assume that individual pharmacokinetic differences cause disparately strong effects of alcohol, *e.g.*, on the brain membranes. It has been suggested that this membrane-disturbing effect represents a primary mechanism of acute alcohol intoxication. Moreover, it was postulated that physical dependence and tolerance can develop as a result of adaptive changes that counteract the membrane-disturbing effect of alcohol. Therefore, individual differences in pharmacokinetic parameters could directly influence the development of metabolic tolerance (20) or indirectly influence the development of physical dependence and tolerance (51).

Central Role of Acetaldehydes

In addition to the direct effects of alcohol many indirect toxic effects are ascribed to the first oxidative metabolite, acetaldehyde (44, 46, 85). The pharmacokinetics of acetaldehyde are characterized by its rate of formation and degradation, as well as the localization of these processes. The rate of formation is obviously identical with the alcohol elimination rate so that a similarly large individual variation is to be expected. Moreover, isoenzymes and enzyme polymorphisms of the main enzyme responsible for the further degradation of acetaldehyde are known.

Acetaldehyde disposal takes place mainly by oxidation to acetate, whereby ALDH plays the major role. In comparison with human ADH surprisingly little is known about human ALDH and its genetic control. Several multiple molecular forms have been described and characterized. These observations make it likely that ALDH activities show an equally large variability as the one found for ethanol oxidation (25, 27, 30, 32, 87). In addition, an enzyme polymorphism found in Oriental populations reveals that about 50% of the individuals lack one enzyme form which seems to be the low K_m mitochondrial enzyme (1, 22, 23, 26). It has recently been shown by immunochemical methods that the enzyme is indeed present, but that it is inactive (31). A very slow acetaldehyde metabolism would be expected in such individuals, and indeed, extremely high acetaldehyde blood and breath levels have been observed as a consequence of this enzyme polymorphism.

Three different states concerning the range of possible blood acetaldehyde levels in humans can be distinguished: (a) the normal range, (b) the "acute aldehyde syndrome" with extremely high acetaldehyde concentrations, and (c) "chronic aldehydism" with only slightly increased blood acetaldehyde levels.

For many years methodologic difficulties have prevented accurate determinations of acetaldehyde concentrations in blood or tissue samples. Now several methods are available which give the various groups of researchers comparable results, and agreement among has been reached on the actual levels (19, 46, 90). The range of normal acetaldehyde concentrations depends on the alcohol dose. With ethanol ingestions of 0.25 to 0.75 gm/kg of body weight, acetaldehyde levels beyond the limit of detection (ca. 0.5 μM) and up to 2 to 3 μM were found.

In individuals suffering from the acute aldehyde syndrome, acetaldehyde concentrations are dramatically elevated to levels of 10 times normal or more. This phenomenon is observed in Orientals lacking the low K_m mitochondrial ALDH (27, 52, 86) or after treatment with ALDH inhibitors such as disulfiram (49). Symptoms such as heavy facial flushing, tachycardia, hypotension, headache, nausea, vomiting, muscle weakness, and sleepiness may occur, and the degree of the symptoms apparently depends on the acetaldehyde levels (67). These symptoms are clearly very aversive and would be expected to prevent individuals from drinking. Factually, only a small percentage of individuals lacking the mitochondrial isoenzyme have been found among Japanese alcoholics as compared with approximately 50% in the whole population (27). This finding is well in keeping with the hypothesis that a genetic hypersensitivity toward the effects of alcohol prevents these individuals from high risk drinking.

In individuals with chronic aldehydism, acetaldehyde concentrations are only slightly elevated to about 2 to 5 times normal (39, 46, 49, 55, 56). Such levels might result from specific isoenzyme patterns of ADH and ALDH and/or the induction of MEOS leading to slightly increased acetaldehyde production combined with a

TABLE 2. DISTRIBUTION OF ALCOHOL DEHYDROGENASE IN HUMAN ORGANS AS DETERMINED BY IMMUNOHISTOCHEMISTRY

Organ	Cells
Liver	Hepatocytes, mainly pericentral
Stomach	Mucosa, mainly parietal-, chief-, mucus-producing cells
Duodenum, Jejunum, Ileum	Mucosa: villus cells
Colon, Rectum	Mucosa: surface epithelium
Pancreas	Islet cells, less in exocrine cells
Thyreoidea	Little (calcitonine producing cells)
Adrenals	Diffuse in cortex and medulla
Prostate	Epithelium
Epididymis	Epithelium
Testes	Seminferous epithelium, Leydig cells
Ovary	Little
Uterus	Little
Kidney	Tubuli epithelium
Heart muscle	Little
Cerebrum	Neurons and astrocytes
Cerebellum	Purkinje cells and astrocytes
Hypothalamus	Neurons

slightly slower removal rate. Higher acetaldehyde levels in the tissue could predispose to heavier intracellular cytotoxic effects, thus leading to increased organic damage. In this context it is of interest that high frequencies of atypical ADH have been observed in alcoholic liver disease (36, 66).

In alcoholic liver disease particularly the mitochondria suffer severe damage. Thus, aldehyde oxidation is disturbed, producing an accumulation of acetaldehyde which in a vicious circle leads to further disturbance of the mitochondrial function (4, 10, 29, 37, 50, 74).

Newer data obtained from activity determinations in needle biopsies suggest that a significant fraction of the acetaldehyde oxidation in man—in contrast to the rat—is localized in the cytoplasm and not exclusively in the mitochondria (34, 61, 62). A comparison of cytosolic and mitochondrial ALDHs revealed that both have a similar K_m for acetaldehyde but that the cytosolic enzyme can also use NADP as a cofactor and in addition is more susceptible to inhibition by disulfiram. These results suggest that acetaldehyde toxicity in man is not based on specific damage to the mitochondria as was postulated for rat liver. More important, however, is the fact that the total oxidation of ethanol to acetate in the cytoplasm leads to an increased NADH to NAD^+ ratio. This in turn could be the reason for the higher susceptibility to alcohol toxicity of man in contrast to the rat, particularly with respect to the development of fatty liver. In rats the continuous prolonged oxidation of ethanol could be a more important pathogenic factor for alcoholic liver damage than acetaldehyde itself (47).

Cytosolic liver ALDH activity is reduced in patients

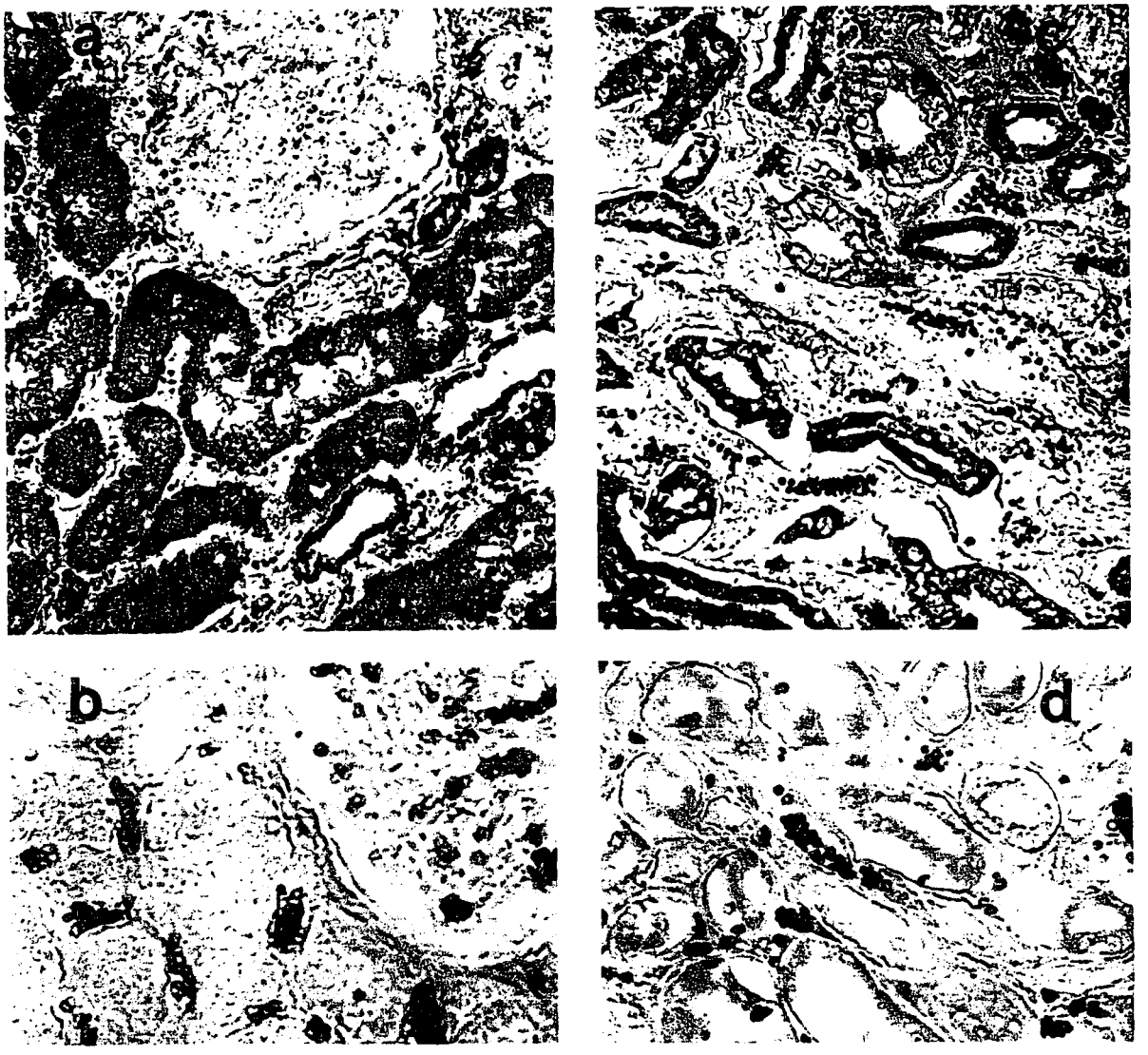

FIG. 5. Immunohistochemical staining of ADH in human kidney. *a* and *c*, sections were incubated with rabbit antihuman ADH antiserum (diluted 1:100 in phosphate-buffered saline). *b* and *d*, controls, incubated with immunoadsorbed antihuman ADH antiserum, depleted of specific anti-ADH antibodies (diluted 1:100 in phosphate-buffered saline). For details refer to reference 7. ×230.

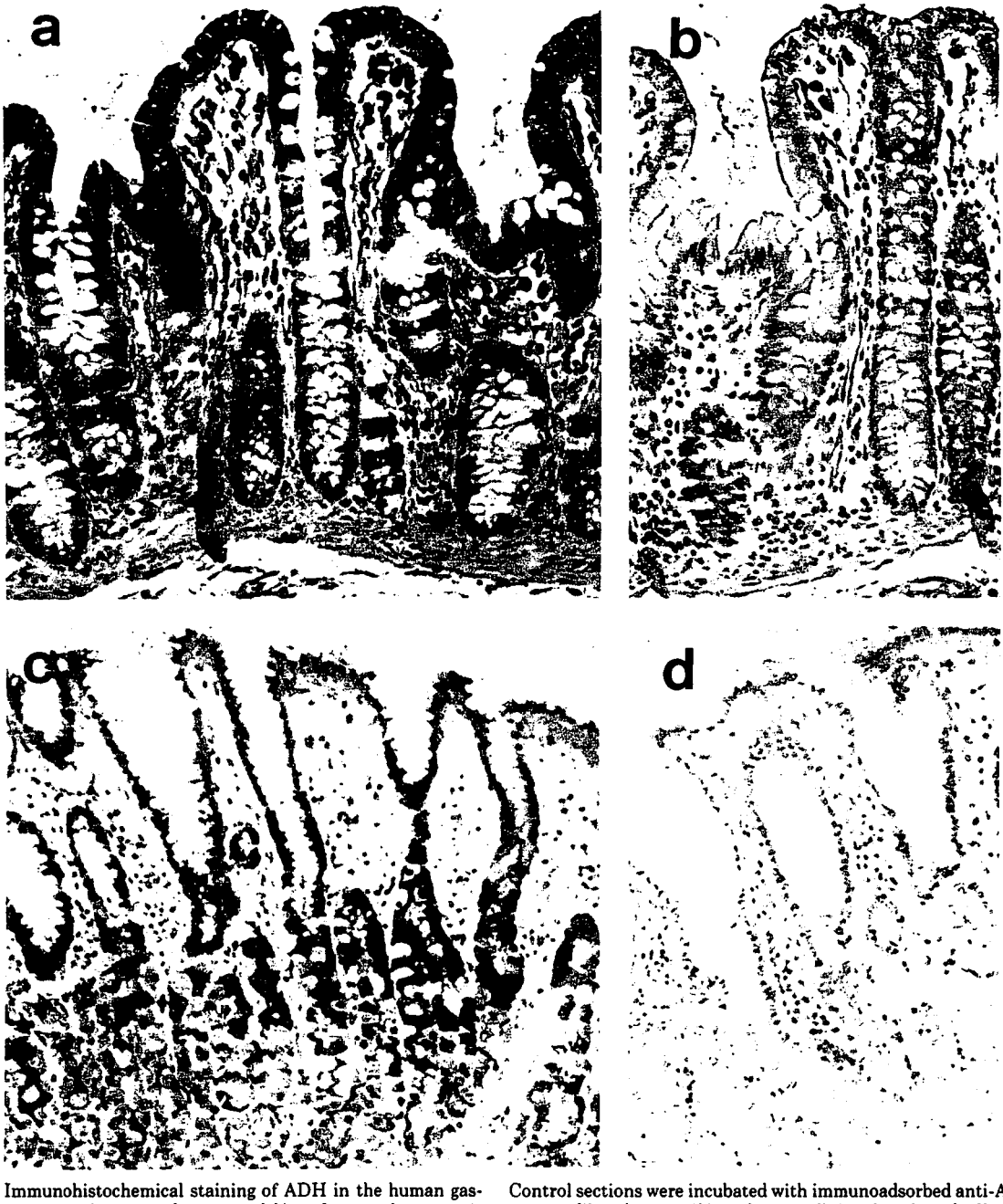

FIG. 6. Immunohistochemical staining of ADH in the human gastrointestinal tract: colon ascendens (*a* and *b*) and stomach corpus (*c* and *d*). Sections were incubated with rabbit antihuman ADH antiserum, diluted 1:100 (*a*) and 1:10 (*c*) in phosphate-buffered saline. Control sections were incubated with immunoadsorbed anti-ADH antiserum, diluted 1:100 (*b*) and 1:10 (*d*) in phosphate-buffered saline. Sections were counterstained with Meyer's hematoxylin. For more details refer to reference 60. Figure 6 *a* and *b*, ×320; *c* and *d*, ×100.

with alcoholic liver disease (33, 54, 55, 75). In these individuals the reduced activity could be the reason for the disturbed acetaldehyde metabolism. Further studies by Peters (61) suggest that the reduced ALDH activity is irreversible, in contrast to reduced ADH activity, suggesting a primary defect. However, this argument is still controversial (78). Acetaldehyde levels were suggested as markers for alcoholism (70). It was shown that a test dose of ethanol led to higher acetaldehyde levels in healthy male relatives of alcoholics. But, as the authors themselves and Eriksson (18) emphasize, the method of blood acetaldehyde determination was not reliable because large amounts of acetaldehyde were produced artificially during sample treatment. It remains questionable whether there is indeed a connection between blood acetaldehyde levels and a predisposition to alcoholism. Preliminary results from analogous acetaldehyde determinations in exhaled air confirm that test persons with

alcoholism in their families had higher acetaldehyde levels after a test dose of ethanol than control individuals (M. A. Schuckit and J. P. von Wartburg, unpublished results).

Acetaldehyde is also known to have its own pharmacologic effects (2), some of which are antagonistic to the direct effects of ethanol. For instance, acetaldehyde can liberate biogenic amines such as catecholamine, thereby counteracting some of the sedative effects of ethanol. Therefore, the overall pharmacologic effects of alcohol in an individual might depend on the equilibrium between direct ethanol and antagonistic acetaldehyde effects. People who produce very little acetaldehyde could thus be more affected by the direct effects of ethanol than those with more acetaldehyde who, after ingestion of identical amounts of alcohol, could show fewer signs of drunkenness due to these antagonistic effects. The relationship between acetaldehyde and ethanol effects could, therefore, determine the inherent tolerance of a person toward the intoxicating effects of alcohol.

ARE THERE TARGET ORGANS?

Many organs including endocrine glands are known to be affected by chronic alcohol intoxication. In some instances it has been observed that acetaldehyde is at least as effective as ethanol. As discussed before, variable concentrations of acetaldehyde in the blood could produce different amounts of this toxic compound diffusing into tissues. On the other hand, the presence of ADH and/or MEOS in such organs could lead to intracellular formation of acetaldehyde. Hence, we undertook an immunohistochemical study of the localization of alcohol dehydrogenase in human tissues (7, 8, 60). Antibodies against a mixture of the pyrazole-sensitive human liver ADH were elicited in rabbits. Protein A-peroxidase with diaminobenzidine as substrate was used to detect anti-ADH bound to the enzyme in tissue thin sections. With this method at least some ADH could be detected in all tissues investigated (Table 2). The control sera were always negative. In some organs certain cell types exhibited much stronger staining than others within the same organ, indicating that the enzyme is unevenly distributed in many organs. Such uneven distribution was found in the kidney (Fig. 5), the gastrointestinal tract (Fig. 6), and also in the liver. This is an important observation because it means that high concentrations of ADH may occur in only a few cells of an organ, although the overall activity of ADH in that tissue may appear as no more than just a trace. High concentrations of acetaldehyde at these specific locations might then lead to discrete cell injury.

In brain, for instance, only some neurons exhibited strong staining in sections of cerebral cortex, cerebellum and hypothalamus. Particularly, some Purkinje cells in the cerebellum stained very strongly (Fig. 7). At this time it is not yet possible to specify the neurons with high

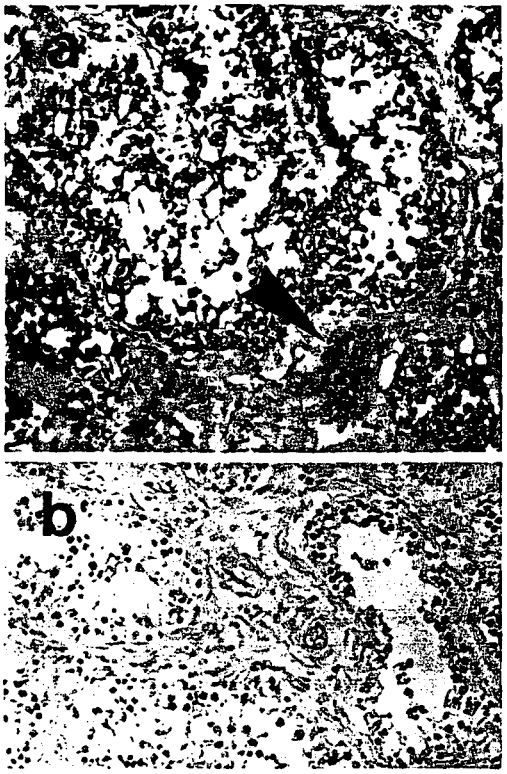

FIG. 8. Immunohistochemical staining of ADH in human testes. *a*, Section was incubated with antihuman ADH antiserum (diluted 1:10 in phosphate-buffered saline). Strongly stained are the seminferous epithelium and the Leydig cells. *Arrowhead* points to a cluster of Leydig cells. *b*, Control, incubated with immunoadsorbed antihuman ADH antiserum (diluted 1:10 in phosphate-buffered saline). Sections were counterstained with Meyer's hematoxylin. For details refer to reference 8. ×250.

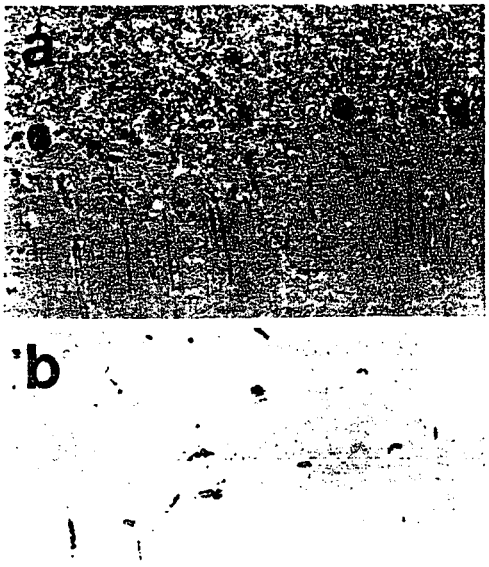

FIG. 7. Immunohistochemical staining of ADH in human cerebellum. *a*, section was incubated with rabbit antihuman ADH antiserum (1:10 diluted in phosphate-buffered saline). Particularly, the Purkinje cells stained strongly for ADH. *b*, Control, incubated with immunoadsorbed antihuman ADH antiserum (diluted 1:10 in phosphate-buffered saline). For details refer to reference 8. ×300.

ADH content, but some neurons also stained in the infundibular stalk of the pituitary gland.

The testes have been shown to be affected by alcohol, and the possible involvement of ADH has been suggested (13, 81, 97). We found most of the ADH in the Leydig cells, followed by the seminiferous epithelium, and least ADH in the interstitial cells (Fig. 8). These results are well in keeping with the proposed role of acetaldehyde formation in that organ. Furthermore, ADH was found in the prostate and in the epididymis.

In the ovary and uterus only weak staining was seen. This observation is again in agreement with the conclusion that ADH plays no role in the toxic effects of ethanol on these organs (63).

In the pancreas not only the exocrine functions but also the endocrine part is known to be affected by alcohol. Interestingly, *in vitro* experiments with rats show that acetaldehyde and acetate have marked effects on insulin and glucagon release (11, 77), and traces of ADH were detected in homogenates of whole pancreatic tissue (14, 69). Although ADH was found in both the exocrine and endocrine parts of the pancreas, much more enzyme was observed in the islets of Langerhans (Fig. 9), suggesting that ADH may be involved in the toxic effects of alcohol on pancreas.

In view of the toxic effects of acetaldehyde on the rat adrenals the possible formation of acetaldehyde from ethanol by ADH would be of special interest. However, the overall staining of the glands was weak, the adrenal cortex showing slightly more ADH than the medulla. Hence, it is more likely that effects of acetaldehyde on the adrenals, such as the release of catecholamines, are mediated by blood acetaldehyde diffusing into the glandular tissue.

In general we may conclude that specialized cells within endocrine organs may contain high amounts of ADH, even though the overall activity of the enzyme in the gland may be quite low. Therefore, many of the organs could be direct targets of acetaldehyde during ethanol intoxication even at low blood acetaldehyde levels.

Acknowledgments: We would like to thank Mrs. Sonja Wyss for her help with the preparation of the manuscript.

Address reprint requests to: Professor Dr. med. J. P. von Wartburg, Medizinisch-chemisches Institut der Universität, Postfach, 3000 Bern 9, Switzerland.

FIG. 9. Immunohistochemical staining of ADH in sections of human pancreas. *a*, Section was incubated with antihuman ADH antiserum (1:10 diluted in phosphate-buffered saline). Depicted is a Langerhans islet which is conspicuously more intensely stained than the surrounding exocrine cells. *b*, Control, depicting the same Langerhans islet as in *a* but incubated with immunoadsorbed antihuman ADH antiserum (diluted 1:10 in phosphate-buffered saline). Sections were counterstained with Meyer's hematoxylin. For details refer to reference 8. ×250.

REFERENCES

1. Agarwal DP, Harada S, Goedde HW: Racial differences in biological sensitivity to ethanol: the role of alcohol dehydrogenase and aldehyde dehydrogenase isozymes. Alcoholism Clin Exp Res 5:12, 1981
2. Akabane J: Aldehydes and related compounds. In Alcohols and Derivatives, International Encyclopedia of Pharmacology and Therapeutics, vol 2, edited by Tremolières J, pp. 523–560. Oxford, Pergamon Press, 1970
3. Bennjon LJ, Li TK: Alcohol metabolism in American Indians and Whites: lack of racial differences in metabolic rate and liver alcohol dehydrogenase. N Engl J Med 294:9, 1976
4. Berstein JD, Penniall R: Effects of chronic ethanol treatment upon rat liver mitochondria. Biochem Pharmacol 27:2337, 1978
5. Bosron, WF, Li TK, Vallee BL: New molecular forms of human liver alcohol dehydrogenase: isolation and characterization of ADH-Indianapolis. Proc Natl Acad Sci USA 77:5784, 1980
6. Bosron WF, Magnes LJ, Li TK: Kinetic and electrophoretic properties of native and recombined isoenzymes of human liver alcohol dehydrogenase. Biochemistry 22:1852, 1983
7. Bühler, R, Hess M, von Wartburg JP: Immunohistochemical localization of human liver alcohol dehydrogenase in liver tissue, cultured fibroblasts and HeLa cells. Am J Pathol 108:89, 1982
8. Bühler R, Pestalozzi D, Hess M, von Wartburg JP: Localization of alcohol dehydrogenase in human tissue: an immunohistochemical study. Biochem Pharmacol Behav 18 (Suppl 1): 55, 1983
9. Bühler R, von Wartburg JP: Purification and substrate specificities of 3 human liver alcohol dehydrogenase isoenzymes. FEBS Lett 144:135, 1982
10. Burke JP, Rubin E: The effects of ethanol and acetaldehyde on the products of protein synthesis by liver mitochondria. Lab Invest 41:393, 1979
11. Calderon-Attas P, Furnelle J, Christophe J: In vitro effects of ethanol and ethanol metabolism in the rat pancreas. Biochim Biophys Acta 620:387, 1980
12. Casier H, Polet H: The metabolism of ethyl alcohol and acetaldehyde labelled with C^{14}. Arch Int Pharmacodyn Ther 120:498, 1959
13. Cicero TJ, Bell RD, Meyer ER, Badger TM: Ethanol and acetaldehyde directly inhibit testicular steroidgenesis. J Pharmacol Exp Ther 213:228, 1980
14. Clemente R, Durand S, Laval J, Thouvenot JP, Ribet A: Métabolisme de l'éthanol par le pancréas de rat. Gastroenterol Clin Biol 1:39, 1977
15. Dubied AM, von Wartburg JP, Bohlken DP, Plapp BV: Characterization and kinetics of native and chemically activated human liver alcohol dehydrogenase. J Biol Chem 252:1464, 1977
16. Edwards JA, Evans PDA: Ethanol metabolism in subjects possessing typical and atypical liver alcohol dehydrogenase. Clin Pharmacol Ther 8:824, 1967
17. Elbel H, Schleyer F: Blutalkohol, pp 1–226. Stuttgart, Georg Thieme Verlag, 1956
18. Eriksson CJP: Elevated blood acetaldehyde levels in alcoholics and their relatives: a reevaluation. Science 207:1383, 1980
19. Eriksson CJP: Human blood acetaldehyde concentration during ethanol oxidation (update 1982). Pharmacol Biochem Behav 18 (Suppl 1):141, 1983

20. Eriksson CJP, Deitrich RA: Metabolic mechanisms in tolerance and physical dependence on alcohol. In The Pathogenesis of Alcoholism, Vol 7, edited by Kissin B, Begleiter H, p 253. New York, Plenum Publishing Co, 1983
21. Farris JJ, Jones BM: Ethanol metabolism and memory impairment in American Indian and White woman social drinkers. J Stud Alcohol 39:1975, 1978
22. Goedde HW, Agarwal DP, Harada S: Pharmacogenetics of alcohol sensitivity. Pharmacol Biochem Behav 18 (Suppl 1):161, 1983
23. Goedde HW, Harada S, Agarwal DP: Racial differences in alcohol sensitivity: a new hypothesis. Hum Genet 51:331, 1979
24. Hanna JM: Metabolic responses of Chinese, Japanese and Europeans to alcohol. Alcoholism 2:89, 1978
25. Harada S, Agarwal DP, Goedde HW: Human aldehyde dehydrogenase: electrophoretic and biochemical studies of 3,4-dihydroxyphenylacetaldehyde metabolizing enzyme and its possible role in alcoholism (abstr). Alcholism Clin Exp Res 6:432, 1982
26. Harada S, Misawa S, Agarwal DP: Liver alcohol dehydrogenase and aldehyde dehydrogenase in the Japanese: isozyme variation and its possible role in alcohol intoxication. Am J Hum Genet 32:8, 1980
27. Harada S, Agarwal DP, Goedde HW, Ishikawa B: Aldehyde dehydrogenase isozyme variation and alcoholism in Japan. Pharmacol Biochem Behav 18 (Suppl 1):151, 1983
28. Harger RN, Hulpieu HR: Extent of absorption of alcohol at various intervals after oral administration. Proc Soc Exp Biol Med 32:1247, 1935
29. Hasumura Y, Teschke R, Lieber CS: Acetaldehyde oxidation by hepatic mitochondria: decrease after chronic ethanol consumption. Science 189:727, 1975
30. Hopkinson DA: Developmental changes and polymorphism in the human aldehyde dehydrogenase isozymes (abstr). Alcholism Clin Exp Res 6:432, 1982
31. Impraim C, Wang G, Yoshida A: Structural mutation in a major human aldehyde dehydrogenase gene results in loss of enzyme activity. Am J Hum Genet 34:837, 1982
32. Inoue K, Lindros KO: Subcellular distribution of human brain aldehyde dehydrogenase (abstr). Alcoholism Clin Exp Res 6:432, 1982
33. Jenkins WJ, Peters TJ: Selectively reduced hepatic acetaldehyde dehydrogenase in alcoholics. Lancet 2:628, 1980
34. Jenkins WJ, Peters TJ: The subcellular localisation of acetaldehyde dehydrogenase in human liver. Cell Biochem Function 1:37–40, 1983
35. Kalant H: Absorption, diffusion, distribution and elimination of ethanol: effects on biological membranes. In The Biology of Alcoholism, Vol 1, Biochemistry, edited by Kissin B, Begleiter H, pp 1–62. New York, Plenum Press, 1971
36. Kamaryt J, Martinek J: Genetically determined alcohol dehydrogenase variants in subjects with liver disease. Scripta Med 52:265, 1979
37. Koch OR, Roatta de Conti LL, Bolanos LP, Stoppani AOM: Ultrastructural and biochemical aspects of liver mitochondria during recovery from ethanol induced alterations: experimental evidence of mitochondrial division. Am J Pathol 90:326, 1978
38. Kopun M, Propping P: The kinetics of ethanol absorption and elimination in twins and supplementary repetitive experiments in singleton subjects. Eur J Clin Pharmacol 11:337, 1977
39. Korsten MA, Matsuzuki S, Feinman L, Lieber CS: High blood acetaldehyde levels after ethanol administration: difference between alcoholic and nonalcoholic subjects. N Engl J Med 292:386, 1975
40. Lamboeuf Y, De Saint Blanquat G, Derache R: Mucosal alcohol dehydrogenase—and aldehyde dehydrogenase—mediated ethanol oxidation in the digestive tract of the rat. Biochem Pharmacol 30:542, 1981
41. Li TK: Enzymology of human alcohol metabolism. Adv Enzymol 46:427, 1977
42. Li TK: Dissociation-recombination studies of ADH-Indianapolis enzyme forms. Alcoholism Clin Exp Res 6:429, 1982
43. Li TK, Magnes LJ: Identification of a distinctive form of alcohol dehydrogenase in human livers with high activity. Biochem Biophys Res Commun 63:202, 1975
44. Lieber CS: Metabolism of ethanol. In Metabolic Aspects of Alcoholism, edited by Lieber CS. Baltimore, University Park Press, 1977
45. Lieber CS, De Carli LM: Hepatic microsomal ethanol-oxidizing system. J Biol Chem 245:2505, 1970
46. Lindros KO: Human blood acetaldehyde levels: with improved methods, a clearer picture emerges. Alcoholism Clin Exp Res 7:70, 1983
47. Lindros KO, Stowell L, Väänänen H, Sipponen P, Lamminsivu U, Pikkarainen P, Salaspuro M: Uninterrupted prolonged ethanol oxidation as a main pathogenic factor of alcoholic liver damage: evidence from a new liquid diet animal model. Liver 3:79, 1983
48. Lundquist F, Wolthers H: The kinetics of alcohol elimination in man. Acta Pharmacol Toxicol 14:265, 1958
49. Maring JA, Weigand K, Brenner HD, von Wartburg JP: Aldehyde oxidizing capacity of erythrocytes in normal and alcoholic individuals. Pharmacol Biochem Behav 18 (Suppl 1):135, 1983
50. Matsuzuki S, Lieber CS: Increased susceptibility of hepatic mitochondria to the toxicity of acetaldehyde after chronic ethanol consumption. Biochem Biophys Res Commun 75:1059, 1971
51. Michaelis EK, Michaelis ML: Physico-chemical interactions between alcohol and biological membranes. In Research Advances in Alcohol and Drug Problems, Vol 7, edited by Smart L, Glaser A, Israel Y, Kalant H, Propham R, Schmidt W, p 127. New York, Plenum Publishing Co, 1983
52. Mizoi Y, Tatsuno Y, Fujiwara S, Kogame M, Fukunaga T, Adachi J, Okada T, Hishida S, Ijiria I: Individual differences of alcohol sensitivity related to polymorphism of alcohol-metabolizing enzymes in Japanese. Pharmacol Biochem Behav 18 (Suppl 1):127, 1983
53. Moser K, Papenberg J, von Wartburg JP: Organverteilung und Heterogenität der Alkoholdehydrogenase bei verschiedenen Spezies. Enzym Biol Clin 9:447, 1968
54. Nautinen H, Lindros K, Pikkarainen P, Salaspuro M: Alcohol and acetaldehyde metabolizing enzymes: their decrease in chronic alcoholics and associated metabolic consequences. Gastroenterology 80:1343, 1981
55. Palmer KR, Jenkins WJ: Impaired acetaldehyde oxidation in alcoholics. Gut 23:729, 1982
56. Palmer KR, Jenkins WJ, Sherlock S: Impaired acetaldehyde oxidation in alcoholism (abstr). Gut 22:419, 1981
57. Pares X, Vallee BL: New human liver alcohol dehydrogenase forms with unique kinetic characteristics. Biochem Biophys Res Commun 98:122, 1981
58. Pequignot G, Tuyns AJ: Compared toxicity of ethanol on various organs. Colloq INSERM 95:17, 1980
59. Pequignot G, Tuyns AJ, Berta JL: Ascitic cirrhosis in relation to alcohol consumption. Int J Epidemiol 7:113, 1978
60. Pestalozzi DM, Bühler R, von Wartburg JP, Hess M: Immunohistochemical localization of alcohol dehydrogenase in the gastrointestinal tract. Gastroenterology, in press, 1983
61. Peters TJ: Studies on the biological basis of alcohol toxicity and pathogenesis of hepatic damage: International Conference on Pharmacological Treatments for Alcoholism. Br J Addict, in press, 1983
62. Peters TJ, Seymour CA: Analytical subcellular fractionation of needle biopsy specimens from human liver. Biochem J 174:635, 1978
63. Pösö J, Pösö AR: Inhibition of rate ovarian ornithine decarboxylase by ethanol in vivo and in vitro. Biochem Biophys Acta 658:291, 1981
64. Reed TE: Racial comparisons of alcohol metabolism: background, problems and results. Alcoholism 2:83, 1978
65. Reed TE, Kalant H, Gibbins RJ, Kapur BM, Rankin JG: Alcohol and acetaldehyde metabolism in Caucasians, Chinese and Amerinds. Can Med Assoc J 115:851, 1976
66. Salaspuro MP, Lindros KO, Nuutinen H: Determinants of blood acetaldehyde in alcoholics and the association of atypical ADH with alcoholic liver cirrhosis in Finnish population. Alcoholism Clin Exp Res 6:436, 1982
67. Sauter AM, Boss D, von Wartburg JP: Reevaluation of the disulfiram: alcohol reaction in man. J Stud Alcohol 38:1680, 1977
68. Schenker TM, Teeple LJ, von Wartburg JP: Heterogeneity and polymorphism of human liver alcohol dehydrogenase. Eur J Biochem 24:271, 1971
69. Schmidt E, Schmidt FW: Enzym-Muster menschlicher Gewebe. Klin Wochenschr 38:957, 1960
70. Schuckit MA, Rayses V: Ethanol ingestion: differences in blood acetaldehyde concentration in relatives of alcoholics and controls. Science 203:54, 1979

71. Smith M, Hopkinson DA, Harris H: Studies on the subunit structure and molecular size of the human alcohol dehydrogenase isozymes determined by the different loci, ADH_1, ADH_2 and ADH_3. Ann Hum Genet 36:401, 1973
72. Smith M, Hopkinson DA, Harris H: Studies on the properties of the human alcohol dehydrogenase isozymes determined by the different loci ADH_1, ADH_2 and ADH_3. Ann Hum Genet 37:49, 1973
73. Southgate HW: The effect of alcohol, under varying conditions of diet, on man and animals with some observations on the fate of alcohol in the body. Biochem J 19:737, 1925
74. Thayer WS, Rubin E: Effects of chronic ethanol intoxication on oxidative phosphorylation in rat liver submitochondrial particles. J Biol Chem 254:7717, 1979
75. Thomas M, Halsall S, Peters TJ: Role of hepatic acetaldehyde dehydrogenase in alcoholism: demonstration of persistent reduction of cytosolic activity in abstaining patients. Lancet 2:1057, 1982
76. Thurman RG, Paschal D, Abu-Murad C, Pekkanen L, Bradford BU, Bullock K, Glassman E: Swift increase in alcohol metabolism (SIAM) in the mouse: comparison of the effect of short-term ethanol treatment on ethanol elimination in four inbred strains. J Pharmacol Exp Ther 223:45, 1982
77. Tiengo A, Valerio A, Molinari M, Meneghel A, Lapolla A: Effect of ethanol, acetaldehyde and acetate on insulin and glucagon secretion in the perfused rat pancreas. Diabetes 30:705, 1981
78. Tipton KF, Henehan GTM, McCrodden JM: Metabolic and nutritional aspects of the effects of ethanol. Biochem Soc Trans 11:59, 1983
79. Wagner JG, Wilkinson PK, Sedman AS, Kay DR, Weidler DJ: Elimination of alcohol from human blood. J Pharm Sci 65:152, 1976
80. Wagner FW, Burger AR, Vallee BL: Kinetic properties of human liver alcohol dehydrogenase: oxidation of alcohols by class I isoenzymes. Biochemistry 22:1857, 1983
81. Van Thiel DH, Gavaler JS, Eagon PK, Chiao YB, Cobb CF, Lester L: Alcohol and sexual function. Pharmacol Biochem Behav 13:125, 1980
82. von Wartburg JP: Studien über die Oxidation von ^{14}C-Aethanol an der Ratte. Inaugural dissertation, Universität Bern, Bern Switzerland, 1959
83. von Wartburg JP: Metabolism of alcohol in normals and alcoholics: enzymes. In The Biology of Alcoholism, Vol 1, Biochemistry, edited by Kissin B, Begleiter H, pp 63–102, New York, Plenum Press, 1971.
84. von Wartburg JP: Alcohol dehydrogenase-dependent alcohol oxidation in the liver and gut. Colloq INSERM 95:221, 1980
85. von Wartburg JP: Alcohol metabolism and alcoholism: pharmacogenetic considerations. Acta Psychiatr Scand 62:179, 1980
86. von Wartburg JP: Comparison of alcohol metabolism in humans and animals. In Animal Models in Alcohol Research, edited by Eriksson K, Sinclair JD, Kiianmaa K, pp 427–443. London, Academic Press, 1980
87. von Wartburg JP: Polymorphism of human alcohol and aldehyde dehydrogenase. In Recent Advances in the Biology of Alcoholism, edited by Stimmel B, Lieber CS, pp 7–23. New York, Haworth Press, 1982
88. von Wartburg JP: Biochemische Auswirkungen der Aethylalkoholeinnahme durch die Mutter auf die Plazenta und den Stoffwechsel im Embryo, Fetus und Neugeborenen. In Child Health and Development, Vol 2, Alcohol and Youth, edited by Jeanneret O, pp 1–13. Basel, S Karger, 1983
89. von Wartburg JP: Pharmacokinetics of alcohol in the normal and alcoholic subject: International Conference on Pharmacological Treatments for Alcoholism. Br J Addict, in press, 1983
90. von Wartburg, JP, Bühler R, Maring JA, Pestalozzi DM: The polymorphism of alcoholism and aldehyde dehydrogenase and their significance for acetaldehyde toxicity. Pharmacol Biochem Behav 18 (Suppl 1):123, 1983
91. von Wartburg JP, Papenberg J, Aebi H: An atypical human alcohol dehydrogenase. Can J Biochem 43:889, 1965
92. von Wartburg JP, Schürch PM: Atypical human liver alcohol dehydrogenase. Ann NY Acad Sci 151:936, 1968
93. Wehner HD: Aethanolausschöpfung im integrierten Gewebsraum. Z Rechtsmed 89:105, 1982
94. Widmark EMP: Die theoretischen Grundlagen und die praktische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung. In Fortschritte der naturwissenschaftlichen Forschung, Heft 11, edited by Abderhalden E, p 140. Berlin-Wien, Urban and Schwarzenberg, 1932
95. Wilkinson PK: Pharmacokinetics of ethanol: a review. Alcoholism Clin Exp Res 4:6, 1980
96. Winston GW, Cederbaum AI: NADH-dependent production of oxy radicals by purified components of the rat liver mixed function oxidase system. J Biol Chem 258:1514, 1983
97. Ylikahri RH, Huttunen MO, Harkonen J: Hormonal changes during alcohol intoxication and withdrawal. Pharmacol Biochem Behav 13:131, 1980
98. Yuki T, Bradford BU, Thurman RG: Role of hormones in the mechanism of the swift increase in alcohol metabolism in the rat. Pharmacol Biochem Behav 13 (Suppl 1):67, 1980
99. Zink P, Reinhardt G: Anstiegsgeschwindigkeit und Anstiegszeit der Blutalkoholkurve bei kurzen Trinkzeiten. Blutalkohol 17:400, 1980