Effect of liver disorders on ethanol elimination and alcohol and aldehyde dehydrogenase activities in liver and erythrocytes

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(Received 11 January/17 May 1988; accepted 2 June 1988)

SUMMARY

1. Liver biopsies were performed in healthy control subjects and in subjects with alcoholic and non-alcoholic liver disease in order to examine alcohol dehydrogenase (ADH; EC 1.1.1.1) and aldehyde dehydrogenase [ALDH; aldehyde dehydrogenase (NAD^+); EC 1.2.1.3] activities. Erythrocyte ALDH and ethanol metabolism were also investigated in the same subjects.

2. Fifteen per cent of the subjects studied (seven of 48 subjects tested) presented atypical ADH activity, characterized by elevated activity at pH 7.4 or 8.8 compared with that found in subjects with the usual ADH form. However, the ethanol elimination curves obtained in two subjects with atypical ADH were indistinguishable from the kinetics of the group with normal ADH. Subjects displaying atypical ADH activity showed normal liver and erythrocyte ALDH activities.

3. Considering only the subjects with the normal ADH form, hepatic ADH activity was unaltered in subjects with non-alcoholic liver disease (chronic hepatitis or cirrhosis) and in those with alcoholic steatosis. Subjects with alcoholic hepatitis or alcoholic cirrhosis showed a lower ADH activity compared with the healthy control group.

4. In spite of the changes detected in subjects with alcoholic liver disease, curves of blood ethanol concentration after oral administration of 0.4 g of ethanol/kg were indistinguishable between the alcoholic hepatitis group and the control group.

5. Hepatic ALDH activity, assayed at 300 μmol/l acetaldehyde, was found to be diminished in all liver pathologies investigated, regardless of their aetiology. Nevertheless, erythrocyte ALDH activity was not modified in subjects with non-alcoholic or alcoholic liver disease. As a result of these findings, no relationship was found between hepatic and erythrocyte ALDH.

6. In summary, our data demonstrate that (a) marked modifications in ADH activity, as found in patients with atypical ADH or in subjects with alcoholic liver disease, are not accompanied by parallel alterations in the kinetics of ethanol disappearance, suggesting that ADH activity *per se* does not limit ethanol metabolism *in vivo*, (b) hepatic high-K_m ALDH activity is decreased in patients with liver disease independent of alcoholism, and therefore decreased ALDH activity cannot be considered as a primary defect in alcoholism but as a consequence of liver damage, and (c) erythrocyte ALDH does not reflect hepatic high-K_m ALDH.

Key words: alcohol dehydrogenase, aldehyde dehydrogenase, cirrhosis, hepatitis, steatosis.

Abbreviations: ADH, alcohol dehydrogenase (EC 1.1.1.1); ALDH, aldehyde dehydrogenase [aldehyde dehydrogenase (NAD^+); EC 1.2.1.3].

INTRODUCTION

The principal site for ethanol elimination from the body is the liver [1, 2] where it is metabolized by enzymatic conversion first to acetaldehyde and then to acetate, which is then metabolized peripherally to CO_2 and H_2O. Although alternative pathways exist, in man the two enzymes that predominantly catalyse the sequential oxidation of ethanol to acetate when the ethanol concentration is below 20 mmol/l are alcohol dehydrogenase (ADH; EC 1.1.1.1) and aldehyde dehydrogenase [ALDH; aldehyde dehydrogenase (NAD^+); EC 1.2.1.3]. Both of these enzymes exhibit multiple isoenzymic forms [1, 3–10], and it has been shown that this variation may be responsible for

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individual and racial differences in sensitivity to alcohol [10–12]. An 'atypical' form of ADH, with an optimal pH at a more physiological pH (8.5) than the normal form of the enzyme (optimum 10.8) and higher specific activity, was described some years ago [3, 4]. Due to the high incidence of 'atypical ADH' in Orientals, it was proposed that this enzyme was responsible for this population's increased sensitivity to ethanol [13], although some doubts have recently been cast on this association [14, 15]. In this latter work, the higher sensitivity to ethanol in the Oriental population was associated with their deficiency in the liver low-K_m intramitochondrial isoenzyme of ALDH, which is responsible for their raised blood acetaldehyde levels. In alcoholics there is also a tendency to augmented circulating levels of acetaldehyde after ethanol ingestion, and this effect has been specifically associated with a decrease in the cytosolic (high-K_m) ALDH isoenzyme [16–18], although it has been also recently reported that the low- rather than the high-K_m isoenzyme is the one that is reduced in these patients [19]. Erythrocytes contain an ALDH that is identical with the hepatic cytosolic enzyme [20] and like hepatic activity, it is reduced in drinking alcoholic subjects [21, 22].

In the present study we have attempted to correlate hepatic ADH activity and the kinetics of ethanol elimination after oral ethanol administration in healthy control individuals and in subjects with different alcoholic and non-alcoholic liver diseases. In addition, we have investigated whether liver disease is characterized by specific alterations in the hepatic activities of ADH or ALDH, and whether changes in erythrocyte ALDH parallel those of liver ALDH. To that effect we have studied the kinetics of ethanol elimination after its oral administration as well as the activities of hepatic ADH and ALDH and erythrocyte ALDH in healthy control Spanish subjects, with the normal and atypical forms of ADH, and in Spanish patients with alcoholic steatosis, alcoholic hepatitis, chronic non-alcoholic hepatitis, non-alcoholic cirrhosis or alcoholic cirrhosis.

METHODS

Subjects

The study was performed on 48 Spanish Caucasian subjects. In one initial set of experiments, we used samples of fresh human liver biopsies taken for diagnosis during abdominal surgery (cholecystectomy) in nine patients who were later proved to be histopathologically unaffected.

In a later set of experiments, we used liver biopsies taken for diagnosis from 39 subjects. Of these 16 had alcoholic liver disease (six with alcoholic steatosis, eight with hepatitis but no cirrhosis, and two with an established cirrhosis), 17 had non-alcoholic liver disorders (13 with chronic active hepatitis and four with primary biliary cirrhosis), and six were normal controls. Each of the alcoholic subjects had consumed more than 80 g of ethanol daily for more than 9 years and was drinking at the time of the study. Subjects were classed as normal controls for the purpose of this study if their liver function and histology tests were normal at the time of the study. Normal subjects or patients with non-alcoholic liver disease had never ingested more than 80 g of ethanol a day. All subjects stopped drinking 1 month before the study. Details of all the above patients are shown in Table 1. An oral ethanol administration test was performed (0.4 g of ethanol/kg of body weight) in some of the same patients in whom the hepatic biopsy was performed.

Determination of ADH and ALDH activities

Immediately after the liver biopsy samples had been excised during abdominal surgery, they were placed in ice-cold 50 mmol/l sodium phosphate buffer (pH 7.5), and after homogenization (1:1, w/v) in a glass Potter vessel with a Teflon pestle, placed in ice and sonicated in a MSE sonifier (set at 12 μ for 1 min). They were then centrifuged at 56000 ***g*** for 20 min at 4°C. The supernatants were then centrifuged at 143000 ***g*** for 60 min, and the final supernatants were immediately used for enzymatic assays.

Liver needle (Trucut) biopsy samples were divided into two so that one portion could be sent for routine histology. The other portion, selected for ADH and ALDH assays, was dropped into ice-cold 50 mmol/l sodium phosphate buffer (pH 7.5). Samples were homogenized with a glass homogenizer in 50 mmol/l phosphate buffer (pH 7.5) (1:1, w/v). Homogenates were spun at 56000 ***g*** for 30 min at 4°C in an ultracentrifuge and the supernatant was used for enzyme assays. ADH and ALDH activities were assayed the same day by the spectrophotometric methods described by Von Wartburg *et al.* [3] and by Blair & Bodley [23] with a few modifications [24].

ADH was assayed at 25°C in cuvettes containing 67 mmol/l sodium phosphate (pH 7.4) or 100 mmol/l glycine–NaOH buffers (pH 8.8 or 10.5), 1.3 mmol/l NAD^+ and 0.8–1.0 mg of protein (20 μl of sample) in 3 ml. The reaction was initiated by adding ethanol up to a final concentration of 17 mmol/l. ALDH assay was performed at 37°C in cuvettes containing 100 mmol/l glycine–NaOH, 130 mmol/l KCl, 1 mmol/l pirazol and 1 mmol/l NAD^+, pH 8.8, and 0.8–1.0 mg of protein in 1 ml. The reaction was started by adding 50 or 300 μmol/l acetaldehyde (final concentration).

When ALDH was assayed in erythrocytes, blood was collected with heparin on the same day that the liver biopsy was taken. Blood was centrifuged at 1000 ***g*** for 10 min and the plasma was discarded. Erythrocytes were washed three times with saline (150 mmol/l NaCl) and successive centrifugations. Washed erythrocytes were sonicated in the presence of 1 mmol/l ethylenediaminetetra-acetate and 0.1% (v/v) mercaptoethanol. After centrifugation at 56000 ***g*** for 20 min at 4°C, a portion of supernatant was passed through a Sephadex C-50 column (at 5°C). The column was washed with 20 mmol/l sodium phosphate buffer (pH 6.0) and portions of the eluate were collected. ALDH activity in the erythrocyte eluates was assayed at 37°C in cuvettes containing 100 mmol/l

Table 1. Clinical data of experimental subjects

Values are shown as ranges.

	Control	Alcoholic steatosis	Alcoholic hepatitis	Alcoholic cirrhosis	Non-alcoholic chronic hepatitis	Non-alcoholic cirrhosis
n	6	6	8	2	13	4
Age (years)	22–63	28–52	31–66	59–61	18–68	27–67
Male/female ratio	5:1	6:0	6:2	1:1	12:1	2:2
History of ethanol consumption (years)	0–5	9–30	10–25	30–40	0–20	0–15
Amount of ethanol consumed daily (g)	0–80	100–190	80–480	180–210	0–80	0–80
Mean corpuscular volume (μl/cell)	88–124	89–104	98–123	103–113	86–98	95–97
Haemoglobin (g/dl)	1.4–1.6	1.2–1.6	1.1–1.7	1.1–1.2	1.2–1.7	1.0–1.6
Aspartate aminotransferase activity (m-units/ml)	13–65	21–109	50–195	48–95	34–200	36–193
Bilirubin (mg/dl)	0.5–0.8	0.7–2.2	0.9–2.3	1.9	0.4–1.4	0.6–1.1

sodium phosphate buffer, 1 mmol/l NAD^+, pH 7.4, and 200 μl of eluate. The reaction was started by the addition of 20 mmol/l acetaldehyde or propionaldehyde (final concentration). In all the assays, measurements were made with a Beckman DU-8B (Kinetics II) spectrophotometer at 340 nm. On each experimental day, assays for one particular sample were performed in duplicate or triplicate interspersed with other samples; the SEM for each particular sample was never above 3%. One unit of enzymatic activity corresponded to 1 μmol of substrate transformed/min. Protein was determined by the method of Lowry *et al.* [25].

Ethanol administration studies

After the ingestion of ethanol (0.4 g/kg body weight) by patients fasted overnight, blood samples were collected in order to determine the circulating concentrations of ethanol and acetaldehyde. Blood samples were collected in tubes containing 300 μl of 100 mmol/l chloral hydrate (2,2,2-trichloro-1,1-ethanediol). Ethanol and acetaldehyde determinations were always performed on fresh blood samples on the same day that they were collected. The method of Von Wartburg & Ris [26] was followed with some modifications [27]. Immediately after placing blood samples in chloral hydrate, proportions were adjusted to 1:1 (v/v) by weighing the tubes and adding the appropriate amount of chloral hydrate. After thorough mixing at 4°C and centrifugation at 1000 ***g*** for 15 min, 150 μl portions of supernatant were placed in 1 ml glass vials containing 300 μl of saline, 500 μl of 1.5 mmol/l propan-1-ol (internal standard) and 50 μl of 60% (w/v) $HClO_4$, after which the vials were hermetically sealed. External standard vials, containing plasma from untreated subjects supplemented with chloral hydrate, saline and different amounts of ethanol and acetaldehyde, were always run in parallel with blank vials. All vials were subjected to headspace gas chromatography performed with a Perkin-Elmer Sigma 15 apparatus and a Carbowax 1540 column. Temperatures were 60°C for the sample thermostat and 150°C for the injector and detector block. With this procedure, the amounts of ethanol and acetaldehyde recovered from fresh plasma samples were 106% and 98%, respectively.

Statistical analysis

Results are expressed as means ± SEM and statistical comparison among the groups was performed by analysis of variance and further post-hoc *t*-tests. Correlation was studied by the minimum square method.

RESULTS

Ethanol metabolism and ADH and ALDH activities in subjects bearing the atypical form of ADH

The presence of the atypical form of ADH was investigated in samples from liver biopsy in a healthy control group and from other groups with different liver diseases: alcoholic steatosis, alcoholic hepatitis, alcoholic cirrhosis, non-alcoholic chronic hepatitis and non-alcoholic cirrhosis. As reported, subjects with atypical ADH had higher activities at pH 7.4 or 8.8, whereas no significant differences were detected at pH 10.5 (data not shown). Among the 48 subjects investigated, only seven presented atypical ADH, corresponding to 15% of the screened population. Table 2 summarizes the incidence of 'atypical' ADH found in the different hepatopathies studied; no increase in the frequency of this form was found in any specific liver disease.

We examined whether the enhanced ADH activity present in subjects with the atypical form of ADH might lead to a faster rate of ethanol elimination. In order to assess this, we studied the curves of blood ethanol concentrations after the oral ingestion of ethanol (0.4 g/kg body weight) in subjects bearing either the normal or the atypical form of ADH (Fig. 1). Blood ethanol concentrations rose immediately after ingestion and they peaked by 30 min, decreasing slowly afterwards. Blood ethanol concentrations were higher than 5 mmol/l even 3 h after ingestion. No differences were detected between the control group (four individuals) and the group with atypical ADH (two individuals) (Fig. 1). Blood acetaldehyde levels were not detectable in either group investigated at all the time points studied (data not shown).

We also investigated the hepatic and erythrocyte activities of ethanol-oxidizing enzymes in subjects with the normal form and subjects with the atypical form of ADH. The atypical form of ADH was not associated with any modification of hepatic and erythrocyte ALDH activities, and in fact, no differences were detected between groups (Table 3).

Table 2. Incidence of 'atypical' ADH in different hepatopathies

	Frequency
Controls	4/15
Alcoholic steatosis	1/6
Alcoholic hepatitis	0/8
Alcoholic cirrhosis	0/2
Non-alcoholic chronic hepatitis	2/13
Non-alcoholic cirrhosis	0/4
Total	7/48

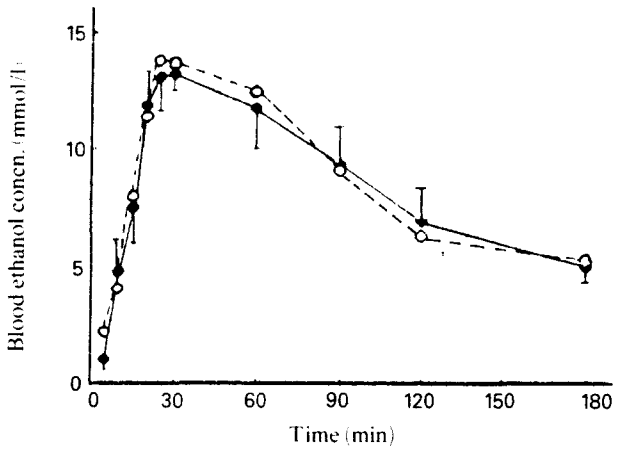

Fig. 1. Changes in blood ethanol concentrations after oral ingestion of 0.4 g of ethanol/kg body weight in subjects with normal (●——●) or atypical (○– – –○) ADH. Results are means ± SEM of four observations in the normal group and two observations in the atypical group. Differences between the groups were not significant.

Effect of alcoholic and non-alcoholic liver disease on hepatic ADH activity and ethanol metabolism

Hepatic ADH activity was investigated in liver needle biopsy samples from a healthy control group and other groups with liver disease. After discarding subjects with 'atypical' ADH, we detected a marked decrease in ADH activity in patients with alcoholic hepatitis and in two subjects with alcoholic cirrhosis (Table 4). Hepatic ADH activity was not altered in any of the other liver diseases studied (Table 4).

The kinetics of ethanol elimination were also investigated in some of the previous groups. In Fig. 2, data on blood ethanol concentrations after oral ingestion (0.4 g/kg) are presented in control and alcoholic hepatitis groups. No significant differences in the pattern of ethanol metabolism were observed between either group, in spite of the lower hepatic ADH activity found in alcoholic hepatitis (see Table 4). Concentrations of circulating acetaldehyde were undetectable or indistinguishable from

Table 4. Hepatic ADH activity in healthy control subjects and patients with alcoholic and non-alcoholic liver disease

Results are means ± SEM for the number of observations given in parentheses. ADH activity was assayed in extracts from liver needle biopsies at pH 8.8, 25°C and 17 mmol/l ethanol. Statistical significance: $*P < 0.05$ compared with controls.

	ADH activity (units/g of liver tissue)
Control	3.39 ± 0.22 (4)
Alcoholic steatosis	3.25 ± 0.52 (5)
Alcoholic hepatitis	2.06 ± 0.29 (8)*
Alcoholic cirrhosis	1.20 (2)
Non-alcoholic chronic hepatitis	2.95 ± 0.24 (11)
Non-alcoholic cirrhosis	3.74 ± 0.65 (3)

Table 3. Liver ADH and ALDH and erythrocyte ALDH activities in subjects with normal and atypical ADH

Results are means ± SEM of 31–38 observations in the normal ADH group and four to five in the atypical ADH group. Liver samples were obtained by needle biopsy. Statistical significance: $*P < 0.05$ compared with the normal ADH group.

	Subjects with normal ADH	Subjects with atypical ADH
Liver ADH (units/g)		
pH 8.8	2.81 ± 0.18	19.01 ± 2.87*
pH 10.5	4.37 ± 0.32	5.13 ± 1.21
Liver ALDH (units/g)		
Low-K_m (50 μmol/l acetaldehyde)	0.46 ± 0.07	0.46 ± 0.09
High-K_m (300 μmol/l acetaldehyde)	0.87 ± 0.09	0.92 ± 0.16
Erythrocyte ALDH (m-units/ml)		
20 mmol/l propionaldehyde	39.8 ± 3.2	40.6 ± 11.1
20 mmol/l acetaldehyde	31.8 ± 3.3	21.8 ± 7.0

zero in both groups. Ethanol elimination was also investigated in the group with alcoholic steatosis, a disorder which is not associated with alterations in hepatic ADH, and again no differences were detected between groups (data not shown). Kinetics of ethanol elimination were not altered in the two cirrhotic patients (one non-alcoholic) that were also studied (data not shown).

Effect of alcoholic and non-alcoholic liver disease on ALDH activities in liver and erythrocytes

The study performed in subjects with liver disease was extended to the investigation of the enzyme responsible for acetaldehyde oxidation, ALDH, in liver and erythrocytes. The results are presented in Table 5. Hepatic ALDH activity was assayed at two different acetaldehyde concentrations, 50 and 300 μmol/l, in order to differentiate between the high- and low-K_m isoenzymes described in human liver [28, 29]. Total (low- plus high-K_m) liver ALDH activity was significantly lower in all groups with liver damage as compared with controls, independent of whether or not they had an alcoholic history. A greater individual variation was found in the low-K_m ALDH activities in liver. Although values were lower in alcoholic and non-alcoholic patients with liver diseases than in controls, the differences were not significant. None of these differences could be accounted for by changes in liver protein concentration as this parameter did not differ among the groups (Table 5). In spite of this variation, liver ADH and ALDH activities among the groups were linearly correlated when individual values were plotted together ($r = 0.576$, $P < 0.01$).

The activity of ALDH in erythrocytes was also studied, when possible, in the same individuals subjected to needle liver biopsy (Table 5). Due to the easy availability of erythrocytes from healthy controls, this group was enlarged with some additional volunteers. ALDH activity was not altered in any of the liver diseases subjected to study, not even in cirrhosis, which manifested greater effects on hepatic ALDH activity. We failed to detect any significant relationship between liver and erythrocyte ALDH activities when individual data from all the groups studied were pooled ($r = 0.039$) (data not shown). There was also no relationship between these two variables when the individual data from just the healthy control group were considered.

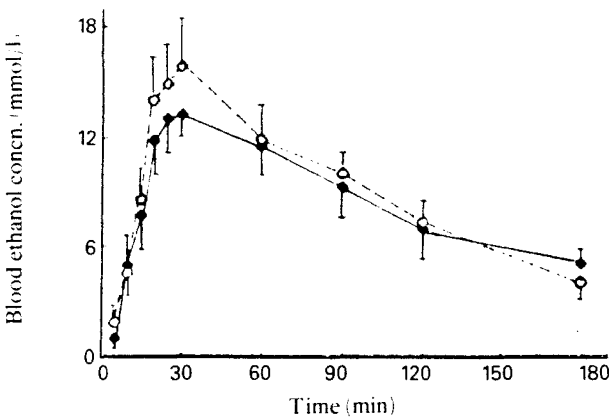

Fig. 2. Changes in blood ethanol concentrations after oral ingestion of 0.4 g of ethanol/kg body weight in healthy control subjects (●——●) and subjects with alcoholic hepatitis (○— — —○). Results are means ± SEM of four observations in both the control group and the group with alcoholic hepatitis. Differences between the groups were not significant.

DISCUSSION

The results of the present study provide evidence that, in humans, modification of ADH activity, such as in subjects with atypical ADH or in patients with alcoholic hepatitis, does not lead to parallel alterations in the kinetics of ethanol elimination. Previous data on ethanol metabolism by subjects with atypical ADH are contradictory. Von Wartburg & Schurch [4] observed a more rapid elimination of alcohol in subjects with atypical ADH, whereas Edwards & Evans [30] found a more rapid elimination in one of two subjects with atypical ADH. In conclusion,

Table 5. Liver and erythrocyte ALDH activity in healthy control subjects and patients with alcoholic and non-alcoholic liver disease

Results are means ± SEM for the number of observations shown in parentheses. ALDH activity was assayed, from extracts of liver needle biopsies, at pH 8.8, 37°C and acetaldehyde concentrations of either 50 (low-K_m) or 300 μmol/l (total). ALDH activity was assayed in extracts of erythrocytes at pH 7.4 and 37°C at an acetaldehyde concentration of 20 mmol/l. Statistical significance: *$P < 0.05$ compared with controls.

	Liver ALDH activity (units/g)		Erythrocyte ALDH activity (m-units/ml)
	Low-K_m	Total	
Controls	0.63 ± 0.17 (4)	1.37 ± 0.17 (5)	31.1 ± 4.5 (15)
Alcoholic steatosis	0.44 ± 0.17 (5)	0.76 ± 0.14 (4)*	31.9 ± 6.9 (3)
Alcoholic hepatitis	0.39 ± 0.07 (7)	0.86 ± 0.10 (7)*	23.5 ± 4.1 (6)
Alcoholic cirrhosis	0.21 (2)	0.34 (2)	44.6 (1)
Non-alcoholic chronic hepatitis	0.40 ± 0.08 (6)	0.77 ± 0.13 (13)*	33.7 ± 5.7 (13)
Non-alcoholic cirrhosis	0.34 (2)	0.79 ± 0.12 (4)*	35.7 ± 12.7 (4)

ADH activity *per se* does not seem to be rate limiting in the pathway of ethanol metabolism. This is in keeping with previous observations suggesting that NADH re-oxidation might be the rate-limiting step in ethanol metabolism [31].

Present results also show that the incidence of the atypical form of hepatic ADH in the Spanish population is in the same range as in other Caucasian populations [3, 11, 12, 14, 30, 31], and the lack of its accumulation in a specific group of patients with liver disease also coincides with previously reported findings [19]. In addition, subjects with atypical ADH studied here have normal ALDH activities, which is an essential difference when compared with Orientals who have a very high incidence of atypical ADH (85–90%) and furthermore a high frequency (52%) of deficiency in the hepatic low-K_m intramitochondrial isoenzymic form of ALDH [14, 15].

In our study we found that patients with alcoholic hepatitis or cirrhosis have reduced liver ADH and ALDH activities, whereas patients with non-alcoholic liver disease or alcoholic steatosis have reduced liver ALDH activity but normal ADH. This latter finding differs from previous reports [19, 32, 33], although the ADH activity in patients with non-alcoholic hepatopathies has been found by Nuutinen [19] to be less affected than in alcoholics. The difference between these reports and our results may be due to the greater liver damage in the former. This possibility is made more likely by the higher liver protein concentration found in hepatopathic patients by Nuutinen [19], whereas we did not find any change. Our findings of reduced liver ALDH activity in both alcoholic and non-alcoholic patients with liver damage contrast with those of Palmer & Jenkins [18, 34], who found no significant decrease in this enzyme activity in patients with non-alcoholic related liver disorders. In agreement with Matthewson *et al.* [32], our results therefore indicate that the abnormality in liver ALDH activity is not specific to alcoholics but occurs in a variety of acute and chronic liver disorders, independent of alcoholism. This abnormality cannot therefore be considered a primary defect in alcoholism or a predisposition factor for alcoholism or alcoholic liver disease as suggested by Thomas *et al.* [17] and, on the contrary, it seems more to be a consequence of liver damage. The linear correlation between hepatic ADH and ALDH activities found here when all the individual values were considered, coincides with the parallel reductions in these two enzymes previously reported in patients with different liver disorders [19, 32, 33]. This linear correlation is even found in other enzymes not directly related to alcohol metabolism such as isocitrate dehydrogenase (NAD^+) (EC 1.1.1.41) [34], indicating that the degree of liver damage may have similar effects on different enzyme activities.

The present findings of a lack of change in erythrocyte ALDH activity among the different groups studied and a lack of a significant correlation between that parameter and hepatic high-K_m ALDH activity, are in contrast to reported reductions in erythrocyte ALDH activity in alcoholic patients [21, 22]. Whether the basis for these contradictory findings stems from differences in the daily ethanol intake by the control subjects or from another factor, remains to be seen. It is known that erythrocyte ALDH activity returns to normal values after a short period of abstinence [19]; this could explain the differences, since our subjects did abstain for at least 1 month. In spite of the kinetic properties, electrophoretic mobility and isoelectric point of erythrocyte ALDH and the hepatic high-K_m enzyme being very similar [20, 35], present findings indicate that erythrocyte ALDH activity is not an adequate marker for the liver enzyme.

In conclusion, our data demonstrate that (a) marked modifications in ADH activity, as found in patients with atypical ADH or in subjects with alcoholic liver disease, are not accompanied by parallel alterations in the kinetics of ethanol disappearance suggesting that ADH activity *per se* does not limit ethanol metabolism *in vivo*, (b) hepatic high-K_m ALDH activity is decreased in patients with liver disease, independent of alcoholism, and therefore decreased ALDH activity must be considered as a consequence of liver damage, not a primary defect in alcoholism, and (c) erythrocyte ALDH does not reflect hepatic high-K_m ALDH.

ACKNOWLEDGMENTS

We thank Dr J. P. Von Wartburg for providing methodological details of enzyme assays, M. Morante for excellent technical work, V. Fresneda for supplying liver biopsies from abdominal surgery, and Carol F. Warren from the ICE of Alcalá de Henares University for help in preparation of the manuscript. This study was supported by a grant from the Comisión Asesora de Investigación Científica y Técnica, Ministerio de Educación y Ciencia of Spain.

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