Biochemical Pharmacology, Vol. 34, No. 9, pp. 1529–1535, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

EFFECTS OF DISULFIRAM ON THE OXIDATION OF BENZALDEHYDE AND ACETALDEHYDE IN RAT LIVER*

EWA HELLSTRÖM-LINDAHL† and HENRY WEINER‡
Department of Biochemistry, Purdue University, West Lafayette, IN 47907, U.S.A.

(*Received* 4 *June* 1984; *accepted* 6 *September* 1984)

Abstract—The *in vitro* oxidation of benzaldehyde and acetaldehyde was studied in liver samples from disulfiram-treated and control rats. With 25 μM substrate, both cytosol and mitochondria appeared to make a nearly equal contribution to the oxidation of benzaldehyde, whereas *ca.* 90% of acetaldehyde oxydation occurred in mitochondria. When the K_m values for benzaldehyde with aldehyde dehydrogenase (ALDH) were determined, two K_m values (3 and 120 μM) were obtained with mitochondria, but only a single K_m value (25 μM) was obtained with the cytosolic fraction. The relatively high K_m (2.9 mM) found with microsomes makes it unlikely that microsomes are important in the oxidation of benzaldehyde. In intact mitochondria, with 200 μM acetaldehyde or benzaldehyde the matrix space enzyme accounted for 77 and 62%, respectively, of the total ALDH activity. When the activity was determined in a mixture containing both substrates, the activity was found not to be additive, indicating that both substrates are oxidized by the same matrix space enzyme. With subcellular fractions, from livers of disulfiram-treated and control rats, a greater degree of inhibition of ALDH was obtained when acetaldehyde was a substrate compared to that with benzaldehyde in cytosol and mitochondria. Microsomal ALDH was not inhibited by disulfiram. In liver slices from rats given disulfiram, a statistically significant inhibition was found when either 25 or 250 μM acetaldehyde was used (46 and 33%). With benzaldehyde, a significant inhibition (24%) was observed only with the lower substrate concentration. Finding that both mitochondrial fractions and slices were less inhibited at the higher substrate concentration implies that the high K_m enzyme is not inhibited. It can be concluded that, in rat, disulfiram inhibiting liver ALDH not only affects oxidation of acetaldehyde, but also that of benzaldehyde.

Aspects of aldehyde metabolism have been investigated for a number of years. The fate of the compounds is either to be oxidized to their corresponding acid by an NAD-dependent aldehyde dehydrogenase (ALDH) or reduced to an alcohol by an NADH-dependent alcohol dehydrogenase or an NADPH-dependent aldehyde reductase prior to excretion.

Aldehydes though seldom ingested can be derived from a variety of endogenous and exogenous sources. Among more common sources are ethanol and biogenic amines which undergo biotransformation to produce aldehydes. Urine contains literally hundreds of acidic compounds, many of which are derived from the oxidation of aldehydes [1].

Inasmuch as ALDH is involved in the oxidation of the aldehyde, an alteration in the level or activity of the enzyme will, in turn, affect aldehyde metabolism. Therapeutically, ALDH is inhibited to deter alcoholics from drinking ethanol. The inhibition of ALDH by drugs such as disulfiram (Antabuse) or citrated calcium cyanamide (Temposil) causes blood acetaldehyde to increase dramatically if a person or animal consumes ethanol [2–5]. What the effect of ALDH inhibition is on other aldehydes is not fully known. It has been shown that the oxidation of the aldehyde derived from dopamine, 3,4-dihydroxyphenylacetaldehyde, is inhibited by disulfiram in both liver and brain of rats [6, 7].

If there were only one form of ALDH in the cell, any inhibition of the enzyme would result in a decreased oxidation rate of all aldehydes. ALDH, however, is found in many subcellular organelles, and furthermore some organelles have several isozymes of the enzyme [8]. Before it could be ascertained that inhibition of metabolism occurs for a particular compound, it would be necessary to know the isozyme responsible for the oxidation of the compound and the ability of that isozyme to be inhibited. Unfortunately, all forms of ALDH have broad substrate specificity. Thus, a simple screening for specificity may not give an answer as to whether or not a particular aldehyde is oxidized *in vivo* by that form of the enzyme.

Toluene is a solvent replacing benzene in many industrial processes for the latter has been found to be carcinogenic [9]. Absorbed toluene is mainly converted by a cytochrome P-450 system to benzyl alcohol, which is further oxidized to benzaldehyde. The next step in the metabolism involves oxidation of benzaldehyde to benzoic acid by an ALDH, followed by conjugation with glycine prior to excretion into urine as hippuric acid [10–12]. In this study we investigate the effects of disulfiram on the *in vitro* oxidation of benzaldehyde in rat liver samples.

* This work was supported in part by Grant AA05842 from the Public Health Service. This is Journal Paper No. 9918 from the Purdue University Agricultural Experiment Station.

† Previous name: Ewa Hellström

‡ Recipient of Research Scientist Development Award KO5 AA00028 from the National Institutes on Alcohol Abuse and Alcoholism. To whom reprint requests should be sent.

MATERIALS AND METHODS

Materials. Disulfiram, gum arabicum, yeast ALDH, horse liver alcohol dehydrogenase, ADP, NAD and NADH were from the Sigma Chemical Co. (St. Louis, MO). Cibacron Blue 3GA covalently attached to an agarose gel was from the Amicon Corp., Scientific Systems Division (Lexington, MA). Acetaldehyde was from the Aldrich Chemical Co. Inc. (Milwaukee, WI) and dissolved in double-distilled water. Benzaldehyde was from Matheson Coleman & Bell (East Rutherford, NJ) and dissolved in acetonitrile. Both aldehydes were freshly distilled before use. All other chemicals were reagent grade and used without further purification.

Animals. Male and female Wistar rats, weighing 250–350 g, were obtained from the breeding facilities of the Biochemistry Department at Purdue University.

Drug administration. Disulfiram (recrystallized from a water–acetone mixture [13]) was suspended in 5% (w/w) gum arabicum (Acacia) and given intraperitoneally in a dose of 150 mg/kg 18 hr before sacrifice. Control rats received the corresponding volumes of 5% gum arabicum.

Subcellular fractionation of rat liver. The rats were killed by cervical dislocation and the livers were quickly removed and placed in an ice-cold sucrose medium (pH 7.2) containing 0.25 M sucrose, 0.5 mM EDTA and 5 mM Tris–HCl. The subcellular fractions were isolated from a 10% (w/v) liver homogenate in this sucrose medium by differential centrifugation [14].

Enzyme assay. The activity of ALDH in subcellular fractions was determined fluorometrically at 25° with an Aminco Fluoromicrophotometer by measuring the formation of NADH. The reaction mixture contained 100 mM sodium phosphate (pH 7.5), 0.5 mM NAD, 0.1 mM pyrazole, 5 μM rotenone and 25 or 250 μM acetaldehyde or benzaldehyde in a total volume of 1 ml. The mitochondrial and microsomal fractions were solubilized with sodium deoxycholate [0.5% (w/v) final concentration] shortly before addition to the reaction mixture. The mitochondrial fractions used for assaying activity with 25 μM substrate and for the *in vitro* studies with disulfiram were cooled to ice temperature and sonicated for 2 × 15 sec with a Branson Sonifier cell disruptor (Setting 5) and centrifuged at 4° for 1 hr at 40,000 *g*. The mitochondrial fraction used for determination of K_m was, after sonication and centrifugation as described above, purified by affinity chromatography. The sample was applicated on a Cibacron Blue 3GA column (20 × 1 cm) and eluted with 5 mM NAD. Protein was determined by the method of Lowry *et al.* [15] with bovine serum albumin as a standard.

Liver slice incubations. Livers were quickly removed and placed in ice-cold 0.25 M sucrose medium, pH 7.2. Liver slices, obtained by the use of a Stadie–Riggs tissue slicer, weighed 35–70 mg and were approximately 0.3 mm thick. The slices were placed in 5-ml glass vials containing 1 ml of Krebs improved Ringer II medium, pH 7.5, saturated with 95:5 O_2/CO_2 [16] and fitted with Teflon-coated septum caps. The vials were placed in a 25° shaking water bath, and acetaldehyde or benzaldehyde was injected into vials with a Hamilton syringe through the septum. The final concentrations of the aldehydes were 25 or 250 μM. The reaction was terminated after 10 min by placing the vials on ice, and shortly afterwards a 0.8-ml aliquot of the solution was transferred to a new glass vial and 0.1 ml of 0.6 M $ZnSO_4$ and 0.1 ml of 0.7 M NaOH were added. The precipitate was removed by centrifugation at 1000 *g* for 10 min at 4°. The concentrations of acetaldehyde and benzaldehyde in the supernatant fractions were determined fluorometrically with the use of potassium-activated ALDH from yeast, or with purified beef liver ALDH obtained from this laboratory. The assay mixture contained 100 mM sodium pyrophosphate buffer (pH 9.0), 0.5 mM NAD and 100 mM KCl when yeast ALDH was used. The recovery of acetaldehyde and benzaldehyde from tissue blanks treated immediately with $ZnSO_4$ and NaOH was approximately 90% and nearly 100% in samples without added slices. ALDH activity in the slice was calculated as the rate of aldehyde disappearance during the first 10 min. The rate of aldehyde disappearance was linear with time for at least 30 min. The rate of disappearance was measured in three slices from each rat liver, and the calculated mean value was taken as one observation. The difference in activity between slices from controls and disulfiram-treated rats was the same whether the rate of disappearance per mg protein or per mg of slice was used.

The amounts of benzyl alcohol and ethanol formed during incubation by reduction of benzaldehyde and acetaldehyde, respectively, were determined enzymatically with horse liver alcohol dehydrogenase [17].

Experiments with intact mitochondria. To verify the integrity of the isolated mitochondria, the following tests were made: (1) the coupling of electron transport to oxidative phosphorylation, and (2) the impermeability of the inner mitochondrial membrane to NADH. Both tests were performed with the use of a Yellow Springs Instrument Clark oxygen electrode. Mitochondria (2–3 mg protein) was added to an incubation medium containing 5 mM $MgSO_4$, 10 mM KCl, 0.25 M sucrose, 1 mM EDTA and 10 mM potassium phosphate buffer (pH 7.4). First, the oxygen consumption was recorded after addition of 5 mM pyruvate and malate, and then again after addition of 1 mM ADP. The marked increase in oxygen utilization in the presence of ADP demonstrated good coupling of the electron transport system to oxidative phosphorylation. The second test was performed by first recording oxygen consumption in the presence of ADP and then after addition of 0.1 mM NADH. Finding no increase in oxygen consumption after adding NADH demonstrated that there was no damage to the mitochondrial inner membrane during isolation of mitochondria. These two tests were performed with each preparation of isolated mitochondria before the incubation experiments were initiated.

The incubation mixture was prepared in 5-ml glass vials with Teflon-coated septum caps and contained mitochondria (2–3 mg protein), 1 mM ADP and incubation medium (saturated with 95:5 O_2/CO_2) in

a total volume of 1.1 ml. Benzaldehyde or acetaldehyde was added with a Hamilton syringe to a final concentration of 200 μM, and the vials were placed in a 25° shaking water bath. The corresponding volume of acetonitrile was added to incubations employing acetaldehyde as the substrate. After a 10-min incubation the reaction was terminated by adding 0.1 ml of 0.6 M $ZnSO_4$ and 0.7 M NaOH. The precipitate was removed by centrifugation at 1000 *g* for 10 min at 4°. The concentrations of benzaldehyde and acetaldehyde in the supernatant fraction, as well as the rate of aldehyde disappearance was determined as described in experiments with rat liver slices. To assay ALDH located in mitochondrial intermembrane space, 5 μM rotenone and 0.5 mM NAD were added to the incubation mixture.

RESULTS

Specific activity and K_m *for benzaldehyde and acetaldehyde in various subcellular organelles.* The K_m for benzaldehyde was determined at a fixed concentration of NAD for samples isolated from three subcellular organelles. Non-linear Lineweaver–Burk plots were obtained when mitochondrial fractions were used. Only a single K_m value was obtained when cytosolic or microsomal fractions were examined. The K_m for benzaldehyde and those previously found for acetaldehyde with the same colony of rats [18] are presented in Table 1. The relatively high K_m (mM) value found with microsomal fractions compared to the μM values found with cytosolic and mitochondrial fractions makes it unlikely that microsomes are important in benzaldehyde oxidation.

Finding the K_m in cytosol just a factor of ten times greater than that of the low K_m mitochondrial isozyme makes it appear likely that cytosolic ALDH may be involved in the oxidation of benzaldehyde. K_m alone, however, cannot be used to predict the contribution of a subcellular organelle to the metabolism. The total activity must be known.

In Table 2 is tabulated the subcellular activity of ALDH for both benzaldehyde and acetaldehyde at two different substrate concentrations. The higher concentration, 250 μM, will not saturate the high K_m enzymes. Knowing the K_m (Table 1) and the velocity at 25 and 250 μM aldehyde allows for the calculation of V_{max}. This V_{max} was really the maximum velocity per mg protein in the crude fraction and not the actual maximal velocity of the pure enzyme. These values are listed in Table 1.

The potential contribution of each organelle to the metabolism can be estimated from the activity found at 25 μM substrate since essentially the same concentration of total protein was found in mitochondria and cytosol [19]. The contribution of microsomes was minimal based on the very high K_m. With acetaldehyde, *ca.* 90% of its oxidation would be calculated to occur in mitochondria using the values in Table 2. With benzaldehyde, both cytosol and mitochondria appear to make nearly an equal contribution to the oxidation.

Oxidation of aldehydes in intact mitochondria. The oxidation of both benzaldehyde and acetaldehyde was studied in isolated mitochondria by monitoring the disappearance of substrates. The results are presented in Table 3. With 200 μM acetaldehyde it was found that 77% of the oxidation can be accounted for by the matrix space enzymes. Essentially the same results were obtained with benzaldehyde, 62% being oxidized in the matrix. Using the V_{max} and K_m data presented in Table 1, it can be calculated that at 200 μM benzaldehyde substrate the low K_m enzyme will perform 43% of the oxidation. With acetaldehyde where the "V_{max}" is 16.4 and 3.4 nmoles/min/mg protein for the high and low K_m systems, respectively, the estimated contribution of the low K_m matrix enzyme was 61%. It cannot be ascertained from this data alone whether or not the same isozyme of ALDH is involved in the oxidation of both benzaldehyde and acetaldehyde.

The rates of oxidation of acetaldehyde and benzaldehyde were measured separately and then determined in a mixture containing both substrates to test if the same ALDH is involved in the metabolism of each substrate. The data are presented in Table

Table 1. K_m and V_{max} of rat liver aldehyde dehydrogenase for benzaldehyde*

Subcellular fraction	Apparent K_m (mM)		V_{max}
	Acetaldehyde†	Benzaldehyde	Benzaldehyde
Mitochondria	1.3	1.2×10^{-1}	1.2
	$<10^{-3}$	2.9×10^{-3}	0.6
	1.3×10^{-4}		
Cytosol	13	2.5×10^{-2}	1.0
	9.4×10^{-1}		
	1.0×10^{-2}		
	2.2×10^{-3}		
	4.5×10^{-3}		
Microsomes	4.0	2.9	5.7

* Assays were performed at 25° in 100 mM phosphate buffer (pH 7.5) as described in Materials and Methods. The V_{max} values are expressed as nmoles NADH formed/min/mg protein and calculated from the K_m data in Table 1 and the velocities at the substrate levels used in Table 2. † Source: Tank *et al.* [18].

Table 2. Activity of rat liver aldehyde dehydrogenase from control and disulfiram-treated animals*

Subcellular fraction	Substrate		ALDH activity (nmoles NADH formed/min/mg protein)		% Inhibition
			Control	Disulfiram	
Mitochondria	Acetaldehyde	25 μM	3.41 ± 0.66	0.74 ± 0.17†	78
		250 μM	6.05 ± 1.72	3.21 ± 0.41‡	47
	Benzaldehyde	25 μM	0.69 ± 0.03	0.38 ± 0.13‡	45
		250 μM	1.35 ± 0.25	1.01 ± 0.15§	25
Cytosol	Acetaldehyde	25 μM	0.34 ± 0.11	0.10 ± 0.03‡	71
		250 μM	0.38 ± 0.10	0.11 ± 0.06†	71
	Benzaldehyde	25 μM	0.44 ± 0.08	0.17 ± 0.09†	61
		250 μM	0.97 ± 0.18	0.54 ± 0.18‡	44
Microsomes	Acetaldehyde	4 mM	4.68 ± 2.16	4.60 ± 1.08	2
	Benzaldehyde	250 μM	0.45 ± 0.10	0.42 ± 0.16	7

* Rats were given disulfiram (150 mg/kg) 18 hr before sacrifice. The activities were assayed in 100 mM phosphate buffer at pH 7.5 as described in Materials and Methods. Values are the means ± S.D. of four to six rats, and statistical significance was determined by Student's *t*-test.
† Significantly different from control values ($P < 0.001$).
‡ Significantly different from control values ($P < 0.01$).
§ Significantly different from control values ($P < 0.05$).

3. Velocity was found not to be additive but was essentially the mean of the separate analysis. This indicated that the same matrix enzyme was involved in the oxidation of each substrate.

Metabolism of aldehydes in rat liver slices. Partially inhibiting ALDH activity and measuring the capacity of the cell to oxidize either substrate could provide information as to the identity of the isozymes responsible for the oxidation of the two aldehydes. The oxidation of both substrates was determined in liver slices isolated from disulfiram-treated rats.

A statistically significant inhibition of acetaldehyde oxidation in slices occurred when either 25 or 250 μM substrate was used. With benzaldehyde, statistically significant inhibition occurred only with the lower concentration (Table 4). Inhibition of acetaldehyde oxidation was greater than that found for benzaldehyde (46% compared to 24%). This can be interpreted as meaning that different isozymes, or at least additional ones, are involved with benzaldehyde oxidation which are not involved in acetaldehyde oxidation. With each substrate a lower degree of inhibition was found when the high substrate concentration was employed. Thus, part of the oxidation of aldehydes must be catalyzed by high K_m enzymes which are uninhibited or less inhibited by disulfiram.

The loss of benzaldehyde by reduction to benzyl alcohol did not make a major contribution to the metabolic rate. At a substrate concentration of 250 μM, it was found that just 12–16% of the benzaldehyde that had disappeared was converted to benzyl alcohol during the course of incubation, presumably by the action of aldehyde reductase [21, 22].

Table 3. Oxidation of acetaldehyde and benzaldehyde by intact rat liver mitochondria*

Substrate	Mitochondria	ALDH activity		
		Matrix space	Intermembrane	Total
Acetaldehyde (200 μM)	1	3.65	1.04	5.17
	2	4.72	1.24	5.54
	3	6.39	2.32	7.41
	4	4.01	1.03	4.47
Benzaldehyde (200 μM)	1	2.33	1.32	3.49
	2	2.66	—	3.37
	3	3.57	2.31	4.27
	4	2.55	1.52	3.64
Acetaldehyde + benzaldehyde	2	3.52 (3.68)		
	3	5.02 (4.97)		

* Intact mitochondria were incubated at 25° for 10 min with 1 mM ADP, substrate and incubation medium, pH 7.4. The activity of the enzyme in the intermembrane space was determined by adding 5 μM rotenone and 0.5 mM NAD to the incubation mixture. Total activity represents the activity in the presence of 0.5 mM NAD. The values in parentheses show the expected values if the two substrates are oxidized by the same matrix enzyme [20]. Activities are expressed as nmoles aldehyde disappeared/min/mg protein (mean of three determinations).

Table 4. Effects of disulfiram on oxidation of acetaldehyde and benzaldehyde in rat liver slices*

Substrate	Oxidation (nmoles aldehyde disappeared/min/mg protein)		% Inhibition
	Controls	Disulfiram	
Acetaldehyde, 25 μM	0.12 ± 0.02	0.065 ± 0.017†	46
Acetaldehyde, 250 μM	0.98 ± 0.16	0.65 ± 0.09†	33
Benzaldehyde, 25 μM	0.20 ± 0.01	0.15 ± 0.02†	24
Benzaldehyde, 250 μM	0.79 ± 0.15	0.69 ± 0.12	13

* Rats were given disulfiram (150 mg/kg) 18 hr before sacrifice. Values are the means ± S.D. of four to six rats.

† Significantly different from control values, $P < 0.01$ (Student's t-test).

Essentially the same degree of reduction was found to occur with 250 μM acetaldehyde, whereas no ethanol could be detected with 25 μM.

Effect of disulfiram inhibition on benzaldehyde and acetaldehyde oxidation in different subcellular organelles. Livers from disulfiram-treated and control animals were fractionated so that three subcellular organelles could be obtained. ALDH activity was measured with both substrates, and the data are presented in Table 2.

A greater degree of inhibition was obtained when acetaldehyde was a substrate compared to that with benzaldehyde in the cytosolic and mitochondrial fractions. With mitochondria, less inhibition was observed when the substrate concentration was increased from 25 to 250 μM. This implies, as previous data presented showed, that high K_m enzyme was less inhibited by the drug than was the low K_m enzyme. Microsomal ALDH was not inhibited by disulfiram.

In all cases, less than total inhibition of ALDH activity was obtained even though a large dose of disulfiram was given to the animal. When disulfiram was added to lyzed control mitochondria, it was found that the degree of inhibition reached a maximum at any disulfiram concentration after 10–12 min of incubation (data not shown). Hence, single 15-min time points were used in these experiments.

As disulfiram concentration increased from 2 to 6 μM, the inhibition of ALDH activity also increased. From the data in Table 5 it can be noted that the percent inhibition obtained was also a function of the substrate concentration in the assay mixture. Higher inhibition was found when lower aldehyde concentrations were used. With 5 μM substrate only the low K_m enzyme was functioning. At 250 μM both the high and low K_m enzymes contributed to the oxidation of substrate. The low K_m enzyme was shown to be responsible for 77 and 62% of acetaldehyde and benzaldehyde oxidation, respectively, at 200 μM substrate (Table 3). Hence, the degree of inhibition of the high K_m enzyme can be estimated from the data obtained at 250 μM substrate presented in Table 5. It can be calculated that virtually no inhibition of the high K_m enzyme occurred.

Table 5. Inhibition of mitochondrial aldehyde dehydrogenase activity *in vitro* by disulfiram*

Disulfiram (μM)	% Inhibition					
	Acetaldehyde (μM)			Benzaldehyde (μM)		
	5	25	250	5	25	250
2	27	30	15 (21)	38	22	16 (24)
4	58	60	48 (45)	65	56	48 (40)
6	81	77	61 (63)	71	63	57 (44)

* Lysed mitochondria were incubated in 100 mM phosphate buffer (pH 7.5) with disulfiram (dissolved in methanol) for 15 min at room temperature before the reaction was initiated by adding 0.5 mM NAD and substrate. Controls with methanol added to the same final concentration were incubated for 15 min. The values in parentheses show the expected values if the high K_m ALDH were not inhibited by disulfiram and are based on the fact that the low K_m enzyme was shown to be responsible for 77 and 62% of acetaldehyde and benzaldehyde oxidation, respectively, at 200 μM substrate. Each experiment was done in duplicate.

DISCUSSION

In rats disulfiram, by inhibiting liver ALDH, not only inhibits acetaldehyde and biogenic aldehyde oxidation but also that of benzaldehyde. If similar inhibition occurs in human liver, then persons taking disulfiram should be cautioned when working with toluene or other compounds which could produce benzaldehyde. Formaldehyde is also oxidized by a matrix-space ALDH [23, 24], in addition to being oxidized by a formaldehyde dehydrogenase system [25]. The fraction of the oxidation catalyzed by each of the enzymes is not known. Thus, by analogy it can be argued that a person taking anti-alcohol drugs could be susceptible to the toxic effect of formaldehyde as well as that of other aldehydes.

Since a person or animal only inhales toluene, the maximum tissue concentration of benzaldehyde would undoubtedly be much lower than found with acetaldehyde after consumption of ethanol. The maximum blood and liver concentrations of acetaldehyde in an alcohol-consuming person are seldom higher than 2 and 20 μM, respectively [26]. In rats it has been shown that in liver the concentration can reach as high as 100 μM [27, 28]. It has been esti-

mated that a person's daily intake of benzaldehyde is 50 mg [29]. Thus, for a 70 kg person the highest body water concentrate would be under 10 μM, assuming no metabolism was taking place. In this study 25 μM benzaldehyde was routinely used, though it is realized that this is a much higher concentration than would be found in a person being exposed to benzaldehyde or toluene. The data obtained at 250 μM are of little physiological significance but were used only to characterize the role of the high K_m ALDH systems.

Inhibitors such as disulfiram have proven useful for studying the role of isozymes of ALDH in the oxidation of acetaldehyde as well as other aldehydes [6, 7, 23, 30–35]. In this study disulfiram was used to help determine the localization of benzaldehyde oxidation in liver. In slice incubation 24% inhibition of benzaldehyde oxidation was found. This is lower than the 46% found with acetaldehyde. The lower degree of inhibition with benzaldehyde can be explained if either different or additional forms of ALDH are involved in its oxidation.

The cumulative data presented in this study make it appear that benzaldehyde was oxidized in mitochondria as well as in cytosol. It was not possible to precisely quantitate the percent of metabolism in each organelle for similar, but not identical, results were obtained when different techniques were employed.

In organelles isolated from livers of disulfiram-fed animals, a 78 and 71% inhibition of acetaldehyde oxidation was found in mitochondria and cytosol, respectively, when 25 μM substrate was used. Yet only 46% inhibition of acetaldehyde metabolism was found in liver slice incubations using disulfiram-treated animals. A similar phenomenon occurred when 25 μM benzaldehyde was the substrate; with slices 24% inhibition was found after disulfiram feeding in contrast to 47 and 61% found in mitochondria and cytosol respectively.

A second major difference between the data obtained with slice incubations and with isolated organelles was found. With mitochondria, acetaldehyde was oxidized at a faster rate than was benzaldehyde. With isolated cytosol the rates were essentially the same when 25 μM substrate was employed. Yet in slices benzaldehyde was oxidized at nearly twice the rate as was acetaldehyde. This could be interpreted as implying that *in vivo* (slices) K_m or V_{max} values may not be the same as those found in isolated organelles or homogenates. An alternative explanation could be that in slices mitochondria is not functioning at maximum capacity due to a lack of oxygen. *If* this be the case *and* benzaldehyde was primarily oxidized in cytosol, it would have appeared that there was more activity with benzaldehyde as a substrate compared to acetaldehyde. The biochemical reasons for the differing results found do not affect the conclusion: disulfiram inhibits oxidation of benzaldehyde.

As also shown by others [14, 18, 23, 24], rat liver mitochondria definitely possess at least two forms of the enzyme. The low K_m form appears to be located in the matrix space and accounts for 32% of the total ALDH activity with benzaldehyde when both enzymes are functioning at their maximal velocity in mitochondria. With 25 μM benzaldehyde the low K_m ALDH accounts for approximately 81% of the activity in mitochondria.

Cytosolic ALDH appears to be important for the oxidation of benzaldehyde, in contrast to what others [23, 24, 28, 30] and now this study found for acetaldehyde. Approximately 40% of the total benzaldehyde oxidizing capacity is located in cytosol, based on the data in Table 2. It is recognized that there may have been some cross contamination of mitochondrial enzymes but the Lineweaver–Burk plots were linear, suggesting that very little mitochondrial ALDH was contaminating the cytosolic fractions.

The fact that cytosolic ALDH was inhibited by difulfiram suggests that compounds that inhibit this form of ALDH may affect metabolism of benzaldehyde but not necessarily acetaldehyde. Thus, it may prove useful to use both aldehydes as substrates when one is testing for chemicals that are possible ALDH inhibitors.

REFERENCES

1. S. I. Goodman and S. P. Markey, *Diagnosis of Organic Acidemia by Gas Chromatography-Mass Spectrometry*. Alan R. Liss, New York (1981).
2. T. M. Kitson, *J. Stud. Alcohol* **38**, 96 (1977).
3. M. D. Faiman, in *Biochemistry and Pharmacology of Ethanol* (Eds. E. Majchrowicz and P. Noble), Vol. 2, p. 325. Plenum Press, New York (1979).
4. J. F. Brien, J. E. Peachey and C. W. Loomis, *Clin. Pharmac. Ther.* **25**, 254 (1979).
5. E. Hellström and O. Tottmar, *Pharmac. Biochem. Behav.* **17**, 1103 (1982).
6. D. Berger and H. Weiner, *Biochem. Pharmac.* **26**, 741 (1977).
7. H. Pettersson and O. Tottmar, *J. Neurochem.* **39**, 628 (1982).
8. H. Weiner, in *Biochemistry and Pharmacology of Ethanol* (Eds. E. Majchrowicz and P. Noble), Vol. 1, p. 107. Plenum Press, New York (1979).
9. J. W. Hayden, R. G. Peterson and J. V. Bruckner, *Clin. Toxic.* **11**, 549 (1977).
10. R. T. Williams, *Detoxication Mechanisms*, 2nd Edn., p. 188. Chapman & Hall, London (1959).
11. M. Ikeda and H. Ohtsuji, *Toxic. appl. Pharmac.* **20**, 30 (1971).
12. K. Cohr and J. Stokholm, *Scand. J. Work Environ. Hlth* **5**, 71 (1979).
13. P. G. Stetcher (Ed.), *The Merck Index*, 8th Edn., p. 393. Merck & Co., Rahway, NJ (1968).
14. O. Tottmar, H. Pettersson and K-H. Kiessling, *Biochem. J.* **135**, 577 (1973).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
16. R. M. C. Dawson, D. C. Elliot, W. H. Elliot and K. M. Jones (Eds.), *Data for Biochemical Research*, p. 507. Oxford University Press, London (1969).
17. H. Krebs, H. A. Freedland, R. Heims and M. Stubbs, *Biochem. J.* **112**, 117 (1969).
18. A. W. Tank, H. Weiner and J. A. Thurman, *Biochem. Pharmac.* **30**, 3265 (1981).
19. H. R. Mahler and E. H. Cordes, *Biological Chemistry*, 2nd Edn, p. 455. Harper & Row, New York (1971).
20. M. Dixon and E. C. Webb, *Enzymes*, 2nd Edn, p. 84. Academic Press, New York (1964).
21. A. W. Tank and H. Weiner, *Biochem. Pharmac.* **28**, 3139 (1979).
22. J. Shultz and H. Weiner, *Biochem. Pharmac.* **28**, 3379 (1979).

23. T. Koivula and M. Koivusalo, *Biochim. biophys. Acta* **397**, 9 (1975).
24. C. Siew, R. A. Deitrich and V. G. Erwin, *Archs Biochem. Biophys.* **176**, 638 (1975).
25. M . Koivusalo and T. Koivula, in *Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Aldo/Keto Reductase* (Eds. H. Weiner and B. Wermuth), p. 155. Alan R. Liss, New York (1982).
26. K. O. Lindros, *Alcoholism: Clin. expl Res.* **6**, 70 (1982).
27. O. Tottmar and H. Marchner, *Acta pharmac. tox.* **38**, 366 (1976).
28. C. J. P. Eriksson and H. Sippel, *Biochem. Pharmac.* **26**, 241 (1977).
29. Flavor and Extract Manufactures Association, *Scientific Literature Review of Benzyl Alcohol, Benzaldehyde, Benzoic Acid and Related Substances in Flavor Usage*, Vol. 1. National Technical Information Service Publn. PB-291-106, U.S. Department of Commerce, Springfield, VA (1978).
30. O. Tottmar and H. Marchner, in *The Role of Acetaldehyde in the Actions of Ethanol*, Satellite Symposium, Sixth International Congress of Pharmacology, Helsinki 1975 (Eds. K. O. Lindros and C. J. P. Eriksson), Vol. 23, p. 47. Finnish Foundation of Alcohol Studies, Helsinki (1975).
31. A. Minegishi, T. Satoh, R. Fukumori, H. Kitagawa and S. Yanaura, *Life Sci.* **24**, 1131 (1979).
32. R. Pietruszko. J. D. Hempel and R. C. Vallari, in *Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Aldo/Keto Reductase* (Eds. H. Weiner and B. Wermuth), p. 61. Alan R. Liss, New York (1982).
33. J. Alderman, C. G. Sanny, E. R. Gordon and C. S. Lieber, in *Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Aldo/Keto Reductase* (Eds. H. Weiner and B. Wermuth), p. 77. Alan R. Liss, New York (1982).
34. E. Hellström and O. Tottmar, *Biochem. Pharmac.* **31**, 3899 (1982).
35. H. Weiner and B. Ardelt, *J. Neurochem.* **42**, 109 (1984).