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COMPARATIVE SUBCELLULAR DISTRIBUTION OF ALDEHYDE DEHYDROGENASE IN RAT, MOUSE AND RABBIT LIVER

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Abstract—The subcellular distribution of hepatic aldehyde dehydrogenase (ALDH) activity was determined in Buffalo, Fischer 344, Long–Evans, Sprague–Dawley, Wistar and Purdue/Wistar rats. These subcellular distributions were compared to the distribution of mouse and rabbit liver ALDH. For the six rat strains, at millimolar propionaldehyde concentrations, NAD-dependent ALDH activity was associated primarily with mitochondria (51%) and microsomes (30%). At millimolar acetaldehyde concentrations, NAD-dependent ALDH was primarily mitochondrial (up to 80%). Less than 1% of total NAD-dependent aldehyde dehydrogenase was found in the cytosol. The highly inbred Purdue/Wistar line possessed significantly less acetaldehyde-NAD ALDH activity as well as less total NADP-dependent ALDH activity than the other strains. In CD-1 mouse liver, millimolar K_m, NAD-dependent ALDH activity was found in mitochondria (60%), microsomes (23%) and cytosol (5%). In rabbit liver, millimolar K_m, NAD-dependent ALDH was also distributed among mitochondria (36%), microsomes (19%) and cytosol (28%). At micromolar substrate concentrations, mitochondria possessed the majority of rat, mouse and rabbit liver ALDH activity. In all three species, NADP-dependent ALDH activity was found predominantly in the microsomal fraction (up to 65%). The cytosol possessed little NADP-dependent ALDH in any species. We conclude that there are significant species differences in the subcellular distribution of aldehyde dehydrogenase between rat, mouse and rabbit liver. In all three species, mitochondria and microsomes possessed the majority of hepatic aldehyde dehydrogenase activity. However, the cytosol of mouse and rabbit liver also made a significant contribution to total ALDH activity. For the six rat strains examined, liver cytosol possessed little or no ALDH activity.

Numerous studies have provided evidence for multiple molecular forms of hepatic aldehyde dehydrogenase (aldehyde NAD(P):oxidoreductase, EC 1.2.1.3 and 1.2.1.5, ALDH) in mammals. In several species, including the cow, horse, sheep and man, distinct mitochondrial and cytosolic isozymes have been identified [1–4]. In other species, including the mouse, rabbit and rat, isozymes of ALDH are found in the microsomal, as well as mitochondrial and/or cytosolic fractions [5–12].

For the mouse and rat, several groups have examined the subcellular distribution of aldehyde oxidation. In the mouse it appears that significant aldehyde dehydrogenase activity is associated with the mitochondrial, microsomal and cytosolic compartments, with both mitochondrial and cytosolic isozymes being implicated in ethanol metabolism [5–7]. A similar situation appears to exist in the rabbit [8–9].

For the rat, there is some debate regarding the subcellular distribution of aldehyde dehydrogenase and the exact physiological roles of the various activities identified. In normal rat liver mitochondria and microsomes, at least three ALDH isozymes can be differentiated on the basis of substrate and coenzyme preferences, kinetics and/or sensitivity to inhibitors [11, 12]. One of the mitochondrial isozymes possesses a K_m for small aliphatic aldehydes in the micromolar range. Most investigators agree that this isozyme is primarily responsible for acetaldehyde oxidation during ethanol metabolism [11–13]. The other mitochondrial and the microsomal isozymes have millimolar substrate K_m values and, though they may function in biogenic aldehyde metabolism [14], their exact physiological roles are largely undefined.

Moreover, considerable disagreement exists regarding the amount and physiological role of aldehyde dehydrogenase in the cytosol. Several groups have reported little or no aldehyde dehydrogenase activity associated with the cytosol in Wistar and Sprague–Dawley strains [11, 12, 15, 16]. Other investigators have reported a relatively low, but significant, contribution by cytosol to total liver rat ALDH activity [14, 17–19]. Both differences in tissue preparation methodology and genetic differences between strains have been proposed to explain the differences in cytosolic ALDH activity reported.

The contribution of the cytosol to basal rat liver aldehyde dehydrogenase activity is complicated by the fact that numerous xenobiotics, including drugs, pesticides and carcinogens, can all induce several additional ALDH activities not detectable in normal liver [20–22]. A common characteristic of these inducible isozymes is that they are almost exclusively

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cytosolic. Also, these inducible activities possess very broad substrate specificities and, except for the isozyme induced by phenobarbital, all utilize $NADP^+$ as efficiently as NAD^+ as coenzyme.

Since little is known of the physiological roles of the "high" K_m mitochondrial and microsomal rat liver ALDHs and the biological basis of ALDH induction by xenobiotics is poorly understood, it seemed appropriate to reexamine, using identical procedures, the subcellular distribution of aldehyde dehydrogenase in several rat strains reported to differ in ALDH activity, as well as in species known to possess significant cytosolic ALDH activity. Such a study should detect any significant differences in ALDH activity between strains, as well as between species. The results of such a study are reported here.

MATERIALS AND METHODS

Materials. Aldehydes and pyrazole were from the Aldrich Chemical Co. NAD^+, $NADP^+$, EDTA, and 2-mercaptoethanol were from the Sigma Chemical Co. Rats of the Buffalo, Fischer 344, Sprague–Dawley and Wistar strains and CD-1 albino mice were from the Charles River Breeding Laboratories. Long–Evans rats were a gift from Dr. R. A. Deitrich of The University of Colorado Medical Center, Denver, CO. Wistar-derived rats maintained as a long-term closed colony at Purdue University were provided by Dr. H. Weiner. Male albino rabbits were originally obtained from a local supplier. All animals were maintained on standard laboratory chow diets and water *ad lib.*

Tissue fractionation. All procedures were performed at 0–4°. At sacrifice, livers were washed and placed in ice-cold 0.25 M sucrose, pH 8.5. A 5-g portion was homogenized for three 1-min intervals at 1100 rpm in 20 ml of 0.25 M sucrose in a Potter–Elvehjem homogenizer. The homogenate was made to 10% (w/v) by addition of 0.25 M sucrose and fractionated as described [12]. The homogenate was centrifuged for 10 min at $400\,g_{max}$, the supernatant fraction was drawn off, and the pellet was washed with 2×25 ml of sucrose. The pellet was resuspended in 10 ml of 60 mM sodium phosphate buffer, pH 8.5, containing 1 mM EDTA and 1 mM 2-mercaptoethanol to yield the nuclear (N) fraction. The pooled supernatant fluid was centrifuged for 10 min at $7500\,g_{max}$, and the pellet was washed twice and finally resuspended in 10 ml of buffer to produce a mitochondrial (M) fraction. The combined supernatant fluid was centrifuged for 10 min at $19{,}000\,g_{max}$, and the pellet was washed twice with sucrose and resuspended in 10 ml of buffer to give the lysosomal (L) fraction. The pooled supernatant fluid was centrifuged at $110{,}000\,g_{max}$ for 60 min, and the surface of the pellet was gently washed with sucrose and resuspended in 10 ml of buffer to yield the microsomal (P) fraction. The final combined supernatant fractions are considered the cytosol (S). The fractions were separated into 5-ml aliquots and frozen at −80°.

Enzyme assays. For enzyme assays, fractions were thawed and made to 0.25% with Triton X-100, incubated on ice for 30 min, and centrifuged at $48{,}000\,g$ for 30 min to remove debris. The resulting clear supernatant fractions were used as enzyme source. All assays were performed at 25° unless otherwise stated.

Aldehyde dehydrogenase activity was determined spectrophotometrically by monitoring the change in A_{340} caused by NADH or NADPH production during the oxidation of aldehyde substrate as described [9, 23]. The reaction mixture contained 1.0 ml of 60 mM sodium phosphate buffer, pH 8.5, containing 1 mM EDTA and 1 mM mercaptoethanol, 1.0 ml of 7.5 mM NAD or NADP in buffer, 0.25 ml of 75 mM propionaldehyde or acetaldehyde or a saturated benzaldehyde solution, unless otherwise noted, 50 or 100 μl of sample, and water to 3.0 ml. When assaying rat or mouse cytosolic fractions, the reaction mixture also contained pyrazole (0.5 mM final concentration). For rabbit, both pyrazole and rotenone (2 μM final concentration) were included in the reaction mixture for the determination of ALDH in each fraction [9]. Monoamine oxidase, acid phosphatase and NADPH–cytochrome *c* reductase were assayed as described by Tottmar *et al.* [11]. Lactate dehydrogenase was determined as described [12]. Proteins were determined by the method of Lowry *et al.* [24] using bovine serum albumin as standard. Activities are expressed as milliunits (1 milliunit = 1 nmole substrate converted/min) per g liver.

RESULTS

In general, the subcellular distribution of aldehyde dehydrogenase was very similar in the six strains of rat examined. In all strains, the mitochondrial and microsomal fractions possessed the vast majority of aldehyde dehydrogenase activity, expressed either as total activity (Tables 1 and 2) or as relative specific activity (Fig. 1A). No consistent, significant differences in activity were observed between the sexes in any strain.

For NAD-dependent aldehyde dehydrogenase, using 6 mM propionaldehyde as substrate, mitochondria and microsomes possessed approximately 51 and 30%, respectively, of the total enzyme activity. The cytosol possessed less than 1% of the total. Mitochondria also possessed the majority of the NAD-dependent ALDH in five of the six strains examined when evaluated as relative specific activity (Fig. 1A). On the same basis, the microsomes of Wistar livers possessed the majority of the NAD-dependent ALDH activity.

Marker enzyme activities (Fig. 1C) indicate that the aldehyde dehydrogenase activity associated with the nuclear fraction was due largely to contamination by mitochondria and membraneous debris. The lysosomal fraction accounted for only a small fraction of the total activity and was likely due to cross-contamination from mitochondria and/or microsomes. Percent recoveries of marker enzymes were greater than 90%. Recovery of ALDH activity routinely exceeded 100% due to complete solubilization of membrane-associated isozymes and apparent aggregation occurring in crude homogenates [12].

With 6 mM or 6 μM acetaldehyde, the majority of NAD-dependent ALDH activity was associated with the mitochondrial fraction (Table 1; Fig. 1A; and

Table 1. Subcellular distribution of millimolar K_m aldehyde dehydrogenase in six strains of rat

Substrate/coenzyme	Fraction	% Total activity*					
		Buffalo	Fischer	Long–Evans	Sprague–Dawley	Wistar	Purdue/Wistar
Propionaldehyde–NAD	Mitochondria	57.5 ± 7.0	50.7 ± 2.2	44.0 ± 2.9	47.2 ± 2.9	50.2 ± 2.0	56.2 ± 2.7
	Microsomes	26.8 ± 1.9	29.9 ± 1.7	33.5 ± 2.8	31.2 ± 2.7	29.4 ± 1.4	29.3 ± 0.8
	Cytosol	0.7 ± 0.0	1.2 ± 0.1	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	1.1 ± 0.1
	Total activity†	3593 ± 302	3781 ± 108	3647 ± 215	3360 ± 232	3126 ± 117	3430 ± 96
Acetaldehyde–NAD	Mitochondria	71.0 ± 8.4	65.9 ± 3.6	56.1 ± 8.3	60.5 ± 6.1	64.9 ± 3.0	80.0 ± 2.6
	Microsomes	17.0 ± 1.9	20.1 ± 1.5	26.2 ± 1.9	26.2 ± 1.7	20.7 ± 1.0	13.9 ± 1.6
	Cytosol	0.2 ± 0.0	0.0	0.0	0.0	0.0	0.0
	Total activity	1279 ± 119	1600 ± 67	1313 ± 152	1243 ± 95	1266 ± 59	703 ± 27‡
Benzaldehyde–NADP	Mitochondria	34.6 ± 2.4	31.9 ± 1.3	23.2 ± 3.2	22.1 ± 3.3	29.3 ± 1.8	39.1 ± 3.2
	Microsomes	41.5 ± 2.4	43.5 ± 4.2	52.8 ± 4.1	56.3 ± 6.8	51.9 ± 3.3	46.8 ± 2.7
	Cytosol	1.4 ± 0.1	2.5 ± 0.2	1.5 ± 0.3	1.7 ± 0.2	1.4 ± 0.2	1.0 ± 0.2
	Total activity	757 ± 32	908 ± 42	952 ± 52	973 ± 48	912 ± 70	426 ± 23‡

* ALDH milliunits/g liver in fraction examined divided by total ALDH milliunits/g liver × 100 ± S.E.M. for six animals of each strain.
† Sum of milliunits/g liver in nuclear, mitochondrial, lysosomal, microsomal and cytosolic fractions ± S.E.M.
‡ Total ALDH activity for this substrate–coenzyme combination in this strain differs significantly from the other strains at at least the $P < 0.05$ level by a Student–Newman–Keuls test.

data not shown). In fact, in the Purdue/Wistar strain, over 80% of the acetaldehyde-oxidizing activity of liver was found in the mitochondria. Under these conditions, no significant aldehyde dehydrogenase activity could be detected in the cytosol of any strain. For five of the six strains, the total liver ALDH activity with acetaldehyde was approximately 33% of that with propionaldehyde (Table 1). For the Purdue/Wistar strain, the acetaldehyde-oxidizing activity was only 20% of that with propionaldehyde.

In five of the six strains, the total NADP-dependent hepatic aldehyde dehydrogenase activity was approximately 25% of the NAD-dependent activity (Table 1). For the Purdue/Wistar strain, total NADP-dependent activity was only about 12% of that with NAD. For all strains, the majority of the NADP-dependent ALDH activity was associated with the microsomal fraction (49 vs 30% for mitochondria). Only about 1% of the NADP-dependent activity was found in the cytosol.

In the CD-1 mouse, mitochondria, microsomes and cytosol each possessed significant aldehyde dehydrogenase activity (Table 2; Fig. 1B). Total mouse liver ALDH was approximately one-half that in the rat. Mitochondria possessed approximately 60% of the propionaldehyde–NAD-dependent activity of mouse liver and microsomes approximately 23%. Aldehyde dehydrogenase in mouse cytosol was almost 5% of the total liver activity, significantly greater than rat cytosol (Table 2; Fig. 1B). At millimolar and micromolar acetaldehyde concentrations, mouse liver mitochondria possessed over 80% of the NAD-dependent ALDH activity. Total mouse liver acetaldehyde-oxidizing activity was about 25% of that with propionaldehyde.

The NADP-dependent aldehyde dehydrogenase activity in CD-1 mouse liver was only about 6% of the propionaldehyde—NAD-dependent activity (Table 2). The NADP-dependent activity was almost equally distributed between mitochondria (40%) and microsomes (37%), with cytosol possessing about 6% of the total (Table 2; Fig. 1B).

Rabbit liver aldehyde dehydrogenase was also localized primarily in the mitochondrial, microsomal and cytosolic fractions but, based on total activity (Table 2) and relative specific activity (Fig. 1), rabbit liver ALDH was clearly distributed differently than that of rat or mouse. The majority of the propionaldehyde–NAD-dependent ALDH was found in the mitochondria (36%), but rabbit cytosol accounted for 28% of the total activity and microsomes approximately 19%. A similar pattern was seen at millimolar and micromolar acetaldehyde concentrations, except that the relative contribution of mitochondria was greater (Fig. 1B and data not shown). The total NAD-dependent activity of rabbit liver was only 17 and 31% that of rat and mouse respectively.

With NADP as coenzyme, microsomes (65%) were the major source of rabbit liver aldehyde dehydrogenase, with less than 10% of the total NADP-dependent activity being associated with the cytosol.

DISCUSSION

We have demonstrated significant differences in

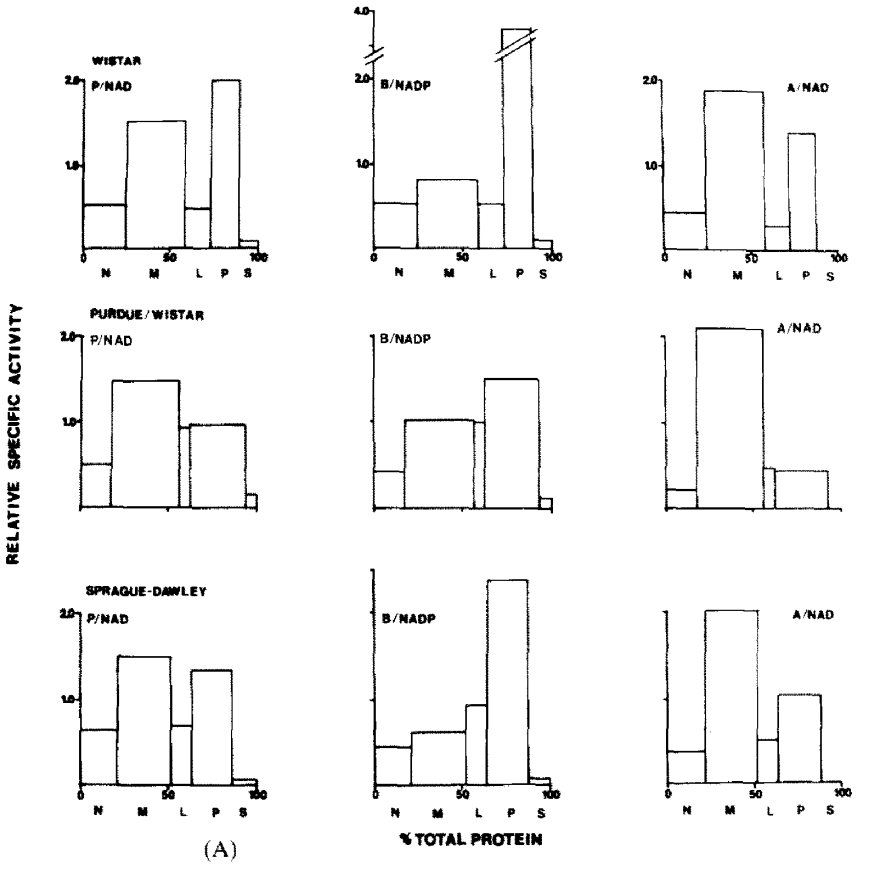

Fig. 1. Aldehyde dehydrogenase activity distribution in rat, mouse and rabbit liver. (A) Subcellular distribution of rat liver ALDH activity. The distribution of ALDH activity in Sprague–Dawley rats was representative of that also found in Buffalo, Fischer 344 and Long–Evans animals. The Wistar and Purdue/Wistar strains differed slightly, but significantly, from the others in subcellular distribution and/or total ALDH activity. (B) Subcellular distribution of mouse and rabbit liver ALDH activity. (C) Marker enzyme activity distributions in rat liver subcellular fractions. Qualitatively identical distributions were obtained for rabbit [9] and mouse liver. Key: subcellular fractions: N, nuclear; M, mitochondrial; L, lysosomal; P, microsomal; S, cytosolic; P/NAD, propionaldehyde–NAD; B/NADP, benzaldehyde-NADP; A/NAD, acetaldehyde-NAD; MAO, monoamine oxidase; ACP, acid phosphatase; CR, NADPH–cytochrome *c* reductase; and LDH, lactate dehydrogenase. Relative specific activity is the ratio of percentage of activity to the percentage of protein in each fraction. The area of each block is proportional to the percentage of total activity found in each fraction, and the height of each block is representative of the degree of purification relative to the initial homogenate.

the subcellular distribution of aldehyde dehydrogenase in rat, mouse and rabbit liver. By utilizing identical tissue fractionation and assay procedures for determining enzyme activity in all three species, we have eliminated the major source of variability inherent in comparing results obtained by different laboratories. Based on the distribution of both marker enzyme and aldehyde dehydrogenase activities, the procedures used provide optimal separation of organelle populations and ALDH in the three species.

A significantly greater proportion of the aldehyde dehydrogenase activity of mouse and, especially, rabbit liver was associated with the cytosol than in the rat, where virtually all the ALDH was associated with particulate fractions. For mouse and rabbit liver cytosolic aldehyde dehydrogenase, the substrate and coenzyme preferences were consistent with the presence of both millmolar and micromolar K_m activities in this fraction. It is unlikely that these activities are artifactual because the distribution of monoamine oxidase, as a marker for outer mitochondrial membrane disruption, and NADPH–cytochrome c reductase, a marker for microsomal membrane fragments, indicates little contamination of rat, mouse or rabbit liver cytosol by leakage from even slightly damaged mitochondria or microsomal membrane fragments. We believe that, *in vivo*, a significant proportion of mouse and rabbit liver ALDH is cytosolic.

The results reported here for rat and rabbit liver aldehyde dehydrogenase are very similar to earlier subcellular distribution studies from this laboratory which employed Sprague–Dawley rats [12] and ran-

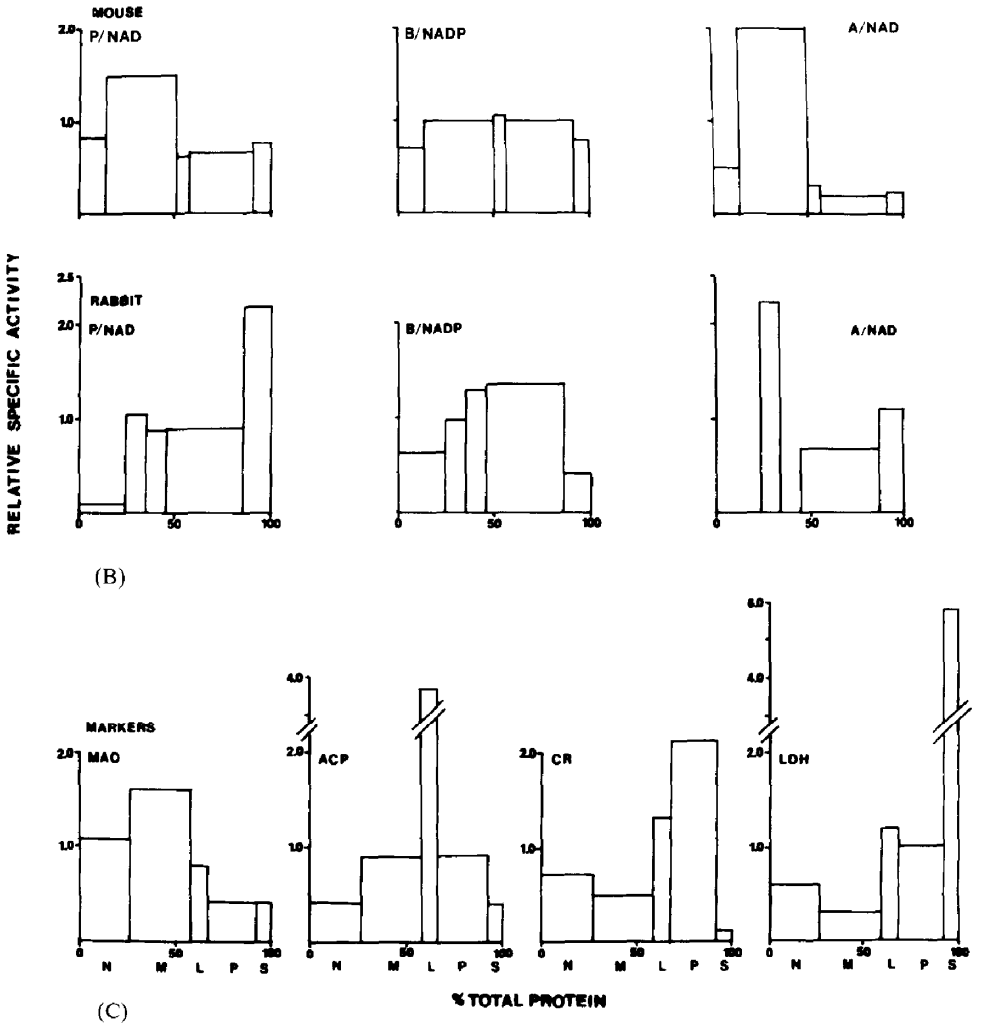

Table 2. Subcellular distribution of aldehyde dehydrogenase in rat, mouse and rabbit liver

Substrate/coenzyme	Fraction	% Total activity*		
		Rat†	Mouse	Rabbit
Propionaldehyde–NAD	Mitochondria	51.0 ± 1.9	59.1 ± 4.1	35.7 ± 3.6
	Microsomes	30.0 ± 0.8	22.8 ± 2.0	19.1 ± 1.7
	Cytosol	0.9 ± 0.1	4.4 ± 0.1	28.4 ± 5.0
	Total	3461 ± 86‡(36)§	1823 ± 138‡(8)	593 ± 20‡(8)
Acetaldehyde–NAD	Mitochondria	66.4 ± 3.1	80.9 ± 7.3	34.9 ± 1.1
	Microsomes	20.7 ± 1.8	8.1 ± 1.0	37.5 ± 12.9
	Cytosol	0.1 ± 0.0	1.6 ± 0.4	27.5 ± 2.7
	Total	1234 ± 109‡(36)	460 ± 39‡(8)	148 ± 7‡,‖(8)
Benzaldehyde–NADP	Mitochondria	29.7 ± 2.5	40.0 ± 6.6	26.2 ± 6.5
	Microsomes	48.8 ± 2.1	37.4 ± 3.2	65.2 ± 10.8
	Cytosol	1.6 ± 0.2	5.6 ± 0.7	8.6 ± 2.0
	Total	817 ± 77‡(36)	117 ± 14(8)	144 ± 8‖(8)

* ALDH milliunits/g liver in fraction divided by total ALDH milliunits/g liver × 100 ± S.E.M.

† Mean percent total activity and milliunits/g liver ± S.E.M. for the six strains examined.

‡ Total ALDH activity for this substrate–coenzyme combination in this species differs significantly from the other species at at least the $P < 0.05$ level by a Student–Newman–Keuls test.

§ Number of livers examined.

‖ Pecent total activity is calculated from the total ALDH activity of the mitochondrial, microsomal and cytosolic fractions only.

dom-bred albino rabbits [9] and which prompted this detailed comparative study. Several additional studies have reported multiple aldehyde dehydrogenase activities in normal rat liver. Of these, Marjanen [15], Tottmar *et al.* [11] and Horton and Barrett [16] reported that essentially all the ALDH activity in Wistar rat liver is due to two to three isozymes located in the mitochondrial and/or microsomal fractions.

Deitrich *et al.* [17], Shum and Blair [18], and Koivula and Koivusalo [19] have reported that, in addition to mitochondrial and/or microsomal isozymes, significant aldehyde dehydrogenase activity is found in the supernatant fraction of Long–Evans or Wistar rat liver. However, reexamination of these studies reveals (1) that enzyme activity was expressed differently than now commonly accepted (nmoles NADH produced/5 min rather than nmoles NADH produced/min [17]), (2) the supernatant fraction was obtained from a low-speed centrifugation (40,000 *g* rather than 110,000 *g* [18]), or (3) the authors ascribed the cytosolic activity to another enzyme activity and/or contamination by other organelles [19].

Nakanishi *et al.* [25] also examined the subcellular distribution of aldehyde dehydrogenase in Long–Evans, Sprague–Dawley and Wistar rats during a study of the induction of ALDH by phenobarbital. Although the three strains differed significantly in their induction response, the basal levels of aldehyde dehydrogenase, especially in the cytosol, were comparable to those reported here.

Therefore, for five of the rat strains examined, even assuming insignificant cross-contamination, the extremely low cytosolic ALDH activity, without detectable acetaldehyde-oxidizing activity, leads us to believe that the contribution of cytosol to rat liver aldehyde dehydrogenase is minimal.

The Purdue/Wistar strain has been maintained as a closed colony without the introduction of new animals for over 30 years. As noted, the distribution of hepatic aldehyde dehydrogenase in these animals differs slightly from that of the other strains examined. Weiner and his colleagues [14, 26, 27] have reported that three to five isozymes of ALDH are identifiable by isoelectric focusing of Purdue/Wistar liver cytosolic fractions. They have also provided evidence that three of these isozymes are encoded by two codominant alleles at a single locus segregating in this population. It is not apparent from these studies, however, what contribution these cytosolic isozymes make to the total ALDH activity of this strain. In the present study, the relative specific activity of Purdue/Wistar cytosolic ALDH was consistently higher than in the other five strains, even though the total activity did not differ significantly. At present, we cannot exclude the possibility that, for this strain, there may be a small contribution of the cytosol to total ALDH activity *in vivo*.

Petersen and colleagues [6, 7] have studied extensively hepatic aldehyde dehydrogenase in several inbred and outbred mouse strains. They have identified NAD-dependent ALDH activity in mitochondria, microsomes and cytosol. However, they find that, expressed as either total or relative specific activity, approximately 60% of the millimolar and of the micromolar K_m ALDH activities is found in the cytosol, with the remainder being distributed among other organelle populations. For NADP-dependent ALDH activity, the subcellular distribution is very similar to that reported here, with respect to both total activity and relative specific activity [7].

Certain minor differences in tissue fractionation procedures and assay conditions can be found. In the present study, the cytosol was composed of the supernatant fractions from both initial organelle separations and subsequent washes; the cytosol prepared by Little and Petersen [7] did not include the washes. They assayed ALDH activity at pH 7.4; our assays were performed at pH 8.5. However, these differences would not appear sufficient to produce such striking differences in subcellular distribution. One difference we cannot explain is that approximately 45% of total protein is associated with the cytosol in the study of Little and Petersen [7]. Allowing for the approximately 4-fold dilution of the original homogenate due to combining all washes, we find about 28% of the total protein to be associated with mouse liver cytosol. The HS mice used by Little and Petersen [7] are a systematically outbred line maintained at The University of Colorado. The CD-1 albino mice employed here are randomly outbred and obtained at regular intervals from the supplier. Since the NADP-dependent aldehyde dehydrogenase activity distribution in both mouse populations appears identical, the possibility that genetic differences are responsible for the observed differences in NAD-dependent ALDH must be examined in detail.

The results here confirm significant differences in the subcellular distribution of rat, mouse and rabbit liver aldehyde dehydrogenase activity. For mouse and rabbit, the mitochondrial, microsomal and cytosolic compartments all make significant contributions to total hepatic ALDH activity. In contrast, rat liver aldehyde dehydrogenase is localized, almost exclusively to the mitochondrial and microsomal fractions. The substrate and coenzyme preferences of the activities present in the various subcellular fractions indicate that multiple forms of ALDH with similar properties are present in all three species.

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