GENETICS OF ALDEHYDE DEHYDROGENASE ISOZYMES IN THE MOUSE: EVIDENCE FOR MULTIPLE LOCI AND LOCALIZATION OF *Ahd-2* ON CHROMOSOME *19*

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ABSTRACT

Electrophoretic and activity variation of the cytoplasmic isozyme of aldehyde dehydrogenase (designated AHD-B_4) was observed among inbred strains and Harwell linkage-testing stocks of *Mus musculus*. The phenotypes are inherited in a normal Mendelian fashion, with two alleles showing co-dominant expression at a single locus (*Ahd-2*). The locus was shown to segregate independently of *Ahd-1* (encoding the mitochondrial AHD-A_2 isozymes on chromosome *4*; HOLMES 1978). Linkage data of *Ahd-2* with *ep* (pale ears), *ru* (ruby eyes) and *bm* (brachymorphic) suggest that it is localized near the centromeric end of chromosome *19*. Electrophoretic evidence for a third AHD isozyme (designated AHD-Cy), which is predominantly localized in the liver microsomal fraction, is also presented.

GENETIC studies on mammalian aldehyde dehydrogenase (AHD; E.C.1.2.1.3) are of particular interest because of its role in ethanol metabolism (PARRILLA *et al.* 1974). AHD catalyzes the second reaction in the oxidation of ethanol in mammalian livers, converting acetaldehyde to acetate. Genetic variation of the enzyme in human livers has recently been reported (HARADA, AGARWAL and GOEDDE 1978), and this polymorphism has been proposed as the molecular basis for individual and racial differences in alcohol sensitivity (GOEDDE, HARADA and AGARWAL 1979).

In mammalian tissues AHD exists as at least three isozymes that are differentially localized in subcellular fractions. A low K_m mitochondrial form and a high K_m cytoplasmic form from mammalian livers have been characterized and shown to be biochemically distinct (TOTTMAR, PETTERSSON and KIESSLING 1973; HORTON and BARRETT 1975; KOIVULO and KOIVUSALO 1975; LINDAHL and FEINSTEIN 1976; SIEW, DEITRICH and IRWIN 1976; ECKFELDT *et al.* 1976; GREENFIELD and PIETRUSZKO 1977). Moreover, evidence for two cytoplasmic isozymes of rat liver AHD, which are differentially induced by drugs and carcinogens, has been reported (DEITRICH, COLLINS and IRWIN 1972; LINDAHL and FEINSTEIN 1976; PETERSEN, COLLINS and DEITRICH 1977); and a membrane-bound enzyme from rat liver microsomes has been purified and shown to have distinctive kinetic and biochemical properties (NAKAYASU, MIHARA and SATO 1978).

Genetic variation of rodent AHD has been previously reported. DEITRICH, COLLINS and IRWIN (1972) have described a gene in the rat (*R*) that controls the inducibility of one of the cytoplasmic isozymes by phenobarbitone. LUSH (1978) and HOLMES (1978) have examined electrophoretic variants for mouse liver AHD among inbred strains of this organism. The latter study demonstrated that the gene (designated *Ahd-1*) encoding the mitochondrial isozyme is localized at the noncentromeric end of chromosome *4*.

This communication describes the electrophoretic variation of the cytoplasmic isozyme of AHD in mouse liver and kidney [designated B_4, since other mammalian liver cytoplasmic ADH isozymes are tetramers (ECKFELDT *et al.* 1976; GREENFIELD and PIETRUSZKO 1977)] occurring among inbred strains and Harwell linkage-testing stocks of *Mus musculus*, which has been used to localize the gene encoding this enzyme (designated *Ahd-2*) on chromosome *19*. Evidence for a third isozyme of AHD that is predominantly localized in the microsomes of liver subcellular fractions is also presented.

MATERIALS AND METHODS

Mouse strains and crosses: 47 inbred strains and 7 Harwell linkage-testing stocks of *Mus musculus* were used in these tudies (Table 1). Two separate series of matings were carried out: (1) matings were made between NZC/Bl females and LR19 males (a Harwell stock designed for positionining genes on chromosome *19*) to obtain F_1 female offspring, which were back-

TABLE 1

Distribution of Ahd-2 *alleles among mouse inbred strains and Harwell linkage-testing stocks*

Ahd-2[a]			*Ahd-2*[b]
A/J	CBA/BrA	129/mA	NZB/Bl
A/BAf	CBA/N		NZC/Bl
A2G/GA	C3H/He	LI†	NZF/A
AKR/Bf	C3H/HeJ	LII†	NZO
AKR/FcRA	C58/J	LIII†	NZW
A/WySwAf	CE/J	LIVA†	NZX
AQR/F16	CCR/A	LVC†	
BALB/c	CSB/Gu	LVI†	
BALB/C/CaA	DD/HeAf	LR 19†	
BALB/C/CrgA	DIR/A		
BALB/c/ByA	FUB/A		
B1022 i (High Line)	GRS/A		
B1022 i (Low Line)	CR/mer		
C57Bl/6J	MAS/A		
C57Bl/Mf	SMJ/J		
C57Bl/Ka	STS/A		
C57Bl/10J	STL/J		
C57Bl/6gA	STL/A		
C57Bl/Imr	SL/NA		
CBA/H	TSI/A		

† Harwell linkage-testing stock.

crossed to LR19 males; the backcross progeny (age 6–8 weeks) were examined for segregation and possible linkage relationship of *Ahd-2* with *ep*, *bm* and *ru* (see Table 2 for details); (2) SM/J females were mated to NZC/Bl males to obtain F_1 female offspring, which were backcrossed to NZC/Bl males; the backcross progeny (age 6–8 weeks) were examined for segregation and possible linkage relationship of *Ahd-1* with *Ahd-2*.

Chemicals: Substrates, co-enzymes and buffer chemicals were purchased from Sigma Chemicals (St. Louis, MO).

Homogenate preparation: Homogenates of tissues of freshly killed animals, as well as large granular, small granular and cytoplasmic subcellular fractions from CSB/Gu mouse liver extracts were prepared as described by HOLMES (1979).

Cellulose acetate electrophoresis and staining: Homogenate supernatants were subjected to zone electrophoresis on Titan III cellulose acetate plates (60 × 75 mm) (Helena Laboratories, TX) with Tris-glycine buffer (25 mM Tris; 192 mM glycine), pH 8.5 (*Ahd-1*) or tris-citrate buffer (100 mM Tris) pH 7.0 (*Ahd-2*, microsomal isozyme of AHD). Different electrophoretic conditions were used: 200 V for 20 min (mitochondrial isozyme of AHD) (A_2)—*Ahd-1*; and 150 V for 60 min (soluble [B_4] and microsomal isozymes of AHD).

The enzymes were stained by an agar-overlay technique that has been described in detail by HOLMES (1978, 1979).

RESULTS

The electrophoretic patterns for AHD isozymes extracted from various tissues of a male 101/H inbred mouse are shown in Figure 1. Three distinct AHD activity zones can be distinguished, as well as several oxidase zones that exhibit activity in the absence of the co-enzyme NAD. The slowest migrating form (designated AHD-B_4) was most active in liver and pancreas extracts, but was also observed in extracts of testis, stomach and lung. The fastest-migrating form (designated AHD-Cy) was observed only in liver extracts; whereas, the intermediate migrating AHD (designated AHD-A_2) was more widely distributed and exhibited highest activities in kidney and liver extracts. The stomach (C_2) isozyme of alcohol dehydrogenase (ADH) was also histochemically stained by this procedure; however, it was distinguished by replacing 20 mM propioaldehyde as substrate with 300 mM ethanol in the presence of the co-enzyme NAD^+ and histochemicals required for ADH staining, and by using electrophoretic variants for the ADH-C_2 isozyme among inbred strains of mice (HOLMES 1979). Subcellular fractionation studies demonstrated that the AHD-Cy isozyme is predominantly localized in the microsomal fraction of buffered isotonic sucrose liver extracts (Figure 2). In contrast, AHD-B_4 is localized only in the cytoplasm;

TABLE 2

Loci examined among backcross individuals from linkage tests with (NZC ♀ × LR19 ♂) F_1 ♀ × LR19 ♂ mice

Locus	Chromosome	LR19	NZC/Bl
pale ears	19	*ep ep*	+ +
ruby eyes	19	*ru ru*	+ +
brachymorphism	19	*bm bm*	+ +
aldehyde dehydrogenase-2	19	*Ahd-2^a Ahd-2^a*	*Ahd-2^b Ahd-2^b*

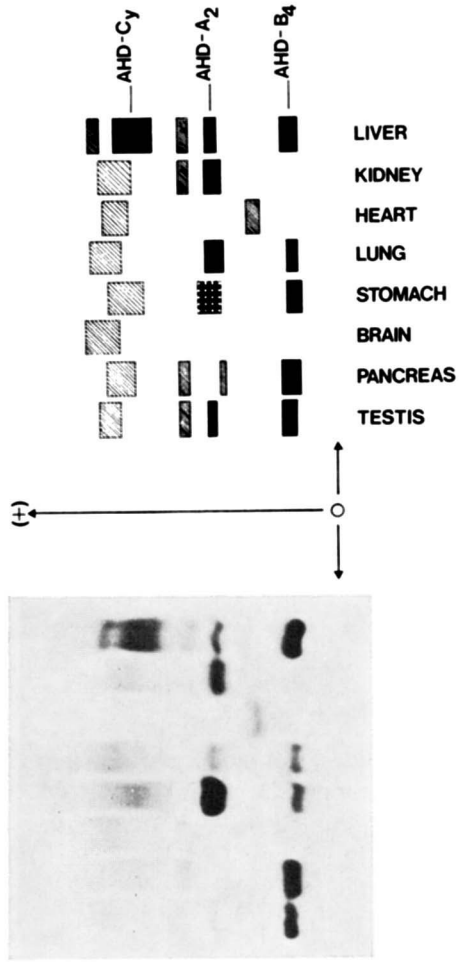

FIGURE 1.—Cellulose acetate zymogram and diagrammatic illustration of mouse aldehyde dehydrogenase (AHD) isozymes from tissue extracts of 101/H inbred mice. AHD activity is represented by ; oxidase activity in the absence of co-enzyme NAD+ is represented by . Alcohol dehydrogenase C_2 isozyme, which exhibits high activity in stomach extracts and also stains with 20 mM propioaldehyde as substrate, is represented by

whereas, AHD-A_2 is predominantly extracted from the large-granule fraction (containing mitochondria, lysosomes and peroxisomes).

A cellulose acetate zymogram illustrating AHD isozymes extracted from livers of NZC/Bl, LR19 and F_1(NZC/Bl × LR19) female mice is shown in Figure 3. Electrophoretic and activity variation was observed for the cytoplasmic AHD-B_4 isozyme; whereas, the large granular (AHD-A_2) and microsomal (AHD-Cy) forms were electrophoretically invariant under the conditions used. LR19 individuals exhibited the phenotype more commonly observed among the mouse inbred strains and Harwell linkage-testing stocks examined (genotype designated *Ahd-2*^{a}); whereas, the alternative phenotype (genotype *Ahd-2*^{b}) was restricted

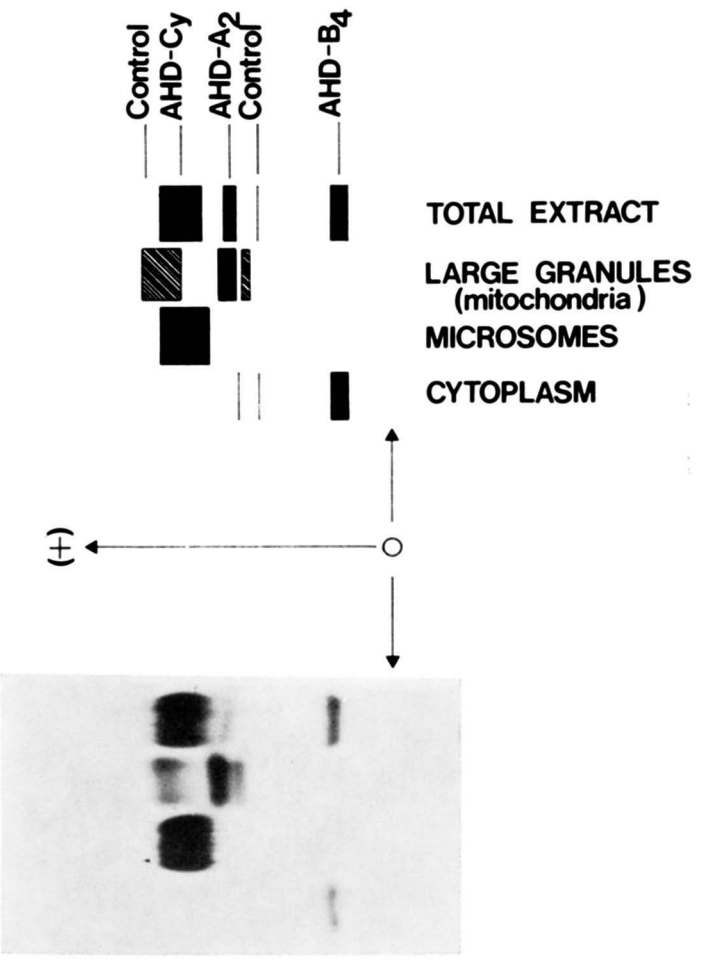

FIGURE 2.—Cellulose acetate zymogram and diagrammatic illustration of mouse liver aldehyde dehydrogenase (AHD) isozymes obtained from subcellular fractionation studies from a CSB/Gu female. AHD activity is represented by ▬; oxidase activity in the absence of the co-enzyme NAD^+ is represented by ▨ or ——. Proposed subunit structures of AHD isozymes are designated at the side of the zymogram.

in its distribution to seven related New Zealand (NZ) inbred strains (Table 1). The NZC/Bl phenotype exhibited a significantly higher level of activity on the zymogram than that of the LR19 phenotype. The hybrid phenotype (*Ahd-2^{a}2^b*) was readily distinguished from the LR19 pattern in both activity and migration pattern, and migrated to an intermediate position on the zymogram, compared with the parental enzymes. Figure 3 also illustrates that the parental allelic isozymes of AHD-B_4 are indeed electrophoretically distinct, since an *in vitro* mixture of the enzymes, when subjected to electrophoresis, reveals two zones of activity.

Seventy-six progeny from a backcross of (SM/J ♀ × NZC/Bl ♂) F_1 ♀ ×NZC/

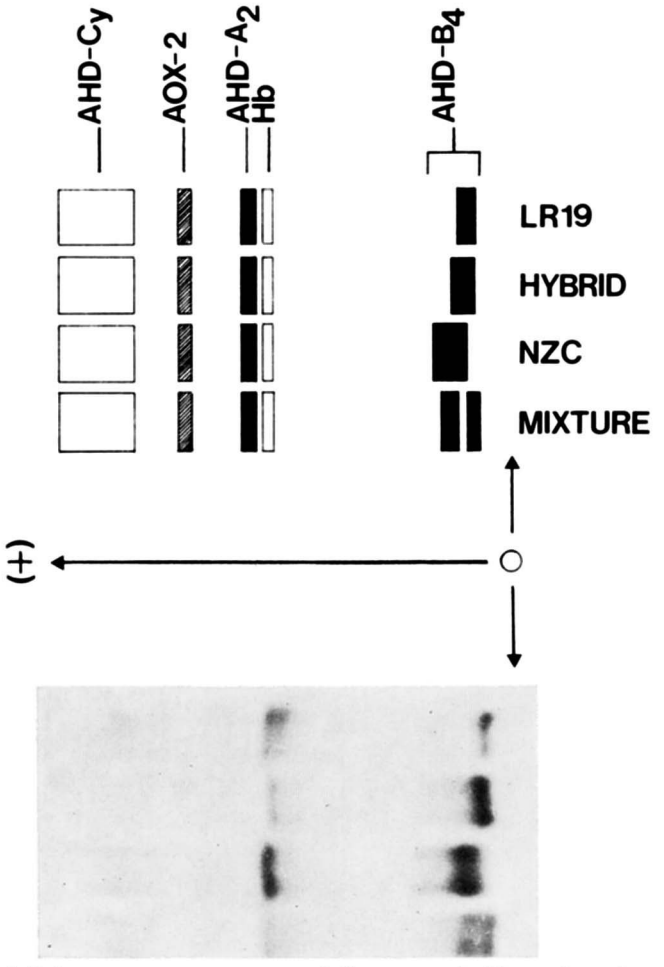

FIGURE 3.—Cellulose acetate zymogram and diagrammatic illustration of mouse liver aldehyde dehydrogenase (AHD) isozymes, AHD-Cy (microsomal), AHD-A_2 (large granular) and AHD-B_4 (cytoplasmic). Genetic variants for AHD-B_4 were observed between NZC/Bl and LR19 inbred mice. Proposed genotypes are *Ahd-2*^{a}*2*^{a}, *Ahd-2*^{b}*2*^{b} and *Ahd-2*^{a}*2*^{b} for the LR19, NZC/Bl and F_1 hybrid animals respectively. Mixture: an *in vitro* mixture (1:1) of LR19 and NZC/Bl liver extracts (20% w/v each diluted 1:3). AOX-2 refers to oxidase activity; Hb designates hemoglobin. Note the higher activity, electrophoretically distinct form of AHD-B_4 in NZC/Bl liver extracts, compared with the phenotype for this isozyme from LR19 mouse liver extract.

Bl ♂ were examined for segregation between the *Ahd-2* and the *Ahd-1* loci. The parental strains of mice exhibited distinct allelic phenotypes for these isozymes: SM/J (*Ahd-2*^{a} *Ahd-2*^{a}) (*Ahd-1*^{b} *Ahd-1*^{b}) and NZC/Bl (*Ahd-2*^{b} *Ahd-2*^{b}) (*Ahd-1*^{a} *Ahd-1*^{a}). The results showed a recombination frequency of 50 ± 5.7%, and the variation observed from the expected 1:1:1:1 ratio for unlinked loci was not significant ($x^2 = 0.42$; 3 df). Since the loci segregate independently, they are not closely localized on a single chromosome in these mice.

TABLE 3

Recombination of Ahd-2 *(aldehyde dehydrogenase-2),* ru *(ruby eyes),* ep *(pale ears) and* bm *(brachymorphic) genes among backcross progeny (NZC/Bl ♀ × LR19 ♂) F_1 ♀ × LR19 ♂ mice*

Parental genotypes

	ru	*ep*	*bm*	*Ahd-2*[a]
F_1	+	+	+	*Ahd-2*[b]
LR 19	*ru*	*ep*	*bm*	*Ahd-2*[a]
	ru	*ep*	*bm*	*Ahd-2*[a]

Progeny classes	*Progeny genotypes* *ru*	*ep*	*bm*	*Ahd-2*	No.	No.
Parental or	*ru/ru*	*ep/ep*	*bm/bm*	a/a	25	56
nonrecombinant	+/*ru*	+/*ep*	+/*bm*	b/a	31	
Single recombinant	+/*ru*	*ep/ep*	*bm/bm*	a/a	1	3
I. *ru-bm*	*ru/ru*	*ep/ep*	+/*bm*	a/a	2	
Single recombinant	*ru/ru*	+/*ep*	*bm/bm*	a/a	1	3
II. *ep-bm*	*ru/ru*	*ep/ep*	+/*bm*	b/a	2	
Single recombinant	+/*ru*	*ep/ep*	*bm/bm*	a/a	0	
III. *ru-ep*	*ru/ru*	+/*ep*	+/*bm*	b/a	0	
Single recombinant	+/*ru*	+/*ep*	+/*bm*	a/a	4	12
IV. *bm-Ahd-2*	*ru/ru*	*ep/ep*	*bm/bm*	b/a	8	
Double recombinant	+/*ru*	*ep/ep*	*bm/bm*	b/a	0	
	ru/ru	*ep/ep*	+/*bm*	*a/a*	0	
	ru/ru	+/*ep*	*bm/bm*	b/a	0	
				Total	72	

Percent recombination

ru-ep	0/72 = 0%	
ru-bm	3/72 = 4.2% ± 2.4%	$\chi^2 = 47.2$; 3df; P~5×10^{-9}
ep-bm	3/72 = 4.2% ± 2.4%	$\chi^2 = 47.2$; 3df; P~5×10^{-9}
bm-Ahd-2	12/72 = 16.7% ± 4.8%	$\chi^2 = 33.4$; 3df; P~1×10^{-5}
ru-Ahd-2	15/72 = 20.8% ± 5.3%	$\chi^2 = 28.3$; 3df; P~1×10^{-5}
ep-Ahd-2	15/72 = 20.8% ± 5.3%	$\chi^2 = 28.3$; 3df; P~1×10^{-5}

Gene order: *Adh-2–bm–(ru–ep)*

Seventy-two progeny from a backcross of (NZC/Bl ♀ × LR19 ♂) F_1 ♀ × LR19 ♂ were examined for the segregation at the *Ahd-2*, *ep* (pale ears), *bm* (brachymorphic) and *ru* (ruby eyes) loci (Table 3). In this cross, both strains of mice exhibited the *Ahd-1*[a] allele for the gene encoding the large granular isozyme, AHD-A_2. Variation from the expected 1:1:1:1:1:1 ratio for unlinked loci was significant for this particular cross ($\chi^2 = 69.25$; 5 df; $P \approx 1 \times 10^{-12}$. Consequently, the results were consistent with these loci being localized on the same chromosome in mice, with a probable gene order *Ahd-2–bm–(ep–ru)* deduced from the recombination frequency data.

DISCUSSION

As a result of this and a previous study (HOLMES 1978), direct genetic evidence is now available for at least two genetic loci for AHD that encode the

mitochondrial and cytoplasmic isozymes in the mouse. These loci have been designated *Ahd-1* and *Ahd-2*, respectively, and it has been proposed that these genes encode the A and B AHD subunits in this organism (HOLMES 1978). This proposal is supported by the following lines of evidence: (1) the independent genetic variation of liver and kidney AHD-A_2 and AHD-B_4 among mouse inbred strains (HOLMES 1978; Table 1); (2) the independent genetic inheritance of these isozymes as demonstrated by the AHD phenotypes observed for progeny of a (C57BL/Go × LVC) ♀ F_1 × LVC ♂ backcross (*Ahd-1* alleles segregating only) (HOLMES 1978), a (NZC/Bl × LR19) F_1 ♀ × LR19 ♂ backcross (*Ahd-2* alleles segregating only) (Table 2; Figure 1) and a (SM/J × NZC/Bl) F_1 ♀ × NZC/Bl ♂ backcross (*Ahd-1* alleles and *Ahd-2* alleles segregating independently); the differential subcellular distribution of these isozymes in mouse liver (HOLMES 1978; Figure 2); and that these isozymes have been purified from other mammalian livers and shown to be biochemically and kinetically distinct isozymes (TOTTMAR, PETTERSSON and KIESSLING 1973; HORTON and BARRETT 1975; KOIVULO and KOIVUSALO 1975; LINDAHL and FEINSTEIN 1976; SIEW, DEITRICH and ERWIN 1976; ECKFELDT *et al.* 1976; GREENFIELD and PIETRUSZKO 1977).

The existence of a third AHD isozyme (designated AHD-Cy, subunit structure not known) in mouse liver extracts is also supported by the results of this present study: (1) the enzyme is found predominantly in the microsomes of liver subcellular fractions, while AHD-A_2 and AHD-B_4 are localized elsewhere in the cell; (2) AHD-Cy is electrophoretically distinct and is observed on cellulose acetate zymograms as a discrete zone only when high ionic strength buffers (*e.g.*, Tris-citrate) are used; (3) the isozyme is predominantly localized in liver, while AHD-A_2 and AHD-B_4 are more widely distributed in mouse tissues (HOLMES 1978; Figure 1); and (4) AHD-Cy is electrophoretically invariant among the inbred strains and Harwell linkage-testing stocks examined. Moreover, NAKAYASU, MIHARA and SATO (1978) have purified a membrane-bound AHD solubilized from rat liver microsomes. Although the isozyme possesses a subunit molecular weight (51,000 daltons) similar to that of cytoplasmic AHD-B_4, it exists in aqueous solution as large polymeric aggregates with molecular weights greater than 360,000. In addition, the microsomal isozyme has a distinct preference for long-chain aliphatic aldehydes, in contrast to the cytosolic isozyme, which exhibits maximum activity with hexanal. Consequently, we propose that there are at least three structural genes for AHD in the mouse, *Ahd-1*, *Ahd-2* and *Ahd-3*, that encode the mitochondrial (A_2), cytoplasmic (B_4) and microsomal (Cy) isozymes in liver extracts.

The linkage studies carried out using a Harwell stock (LR19) homozygous for *ep* (pale ears), *ru* (ruby eyes) and *bm* (brachymorphic) and one of the inbred New Zealand strains (NZC/Bl) that exhibits the variant form of AHD-B_4 (Tables 1 and 3) demonstrated that *Ahd-2* is linked to these recessive genes in the mouse. LANE, EICHER and SOUTHARD (1974) have recently reviewed available evidence concerned with the identification and linear order of the known loci on chromosome *19* and have positioned the centromere on this chromosome,

using cytogenetic methods. The results of this paper and the present communication are consistent with *Ahd-2* being localized at the centromeric end of this chromosome with the following probable order: centromere–*Ahd-2*–*bm*–(*ep*–*ru*).

In summary, the present results provide evidence that at least three genetic loci, *Ahd-1*, *Ahd-2* and *Ahd-3*, are responsible for the mitochondrial, cytoplasmic and microsomal isozymes, respectively, in mouse liver. Genetic variation of the cytoplasmic (B_4) isozyme was used to localize *Ahd-2* on chromosome *19*.

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