About 50% of the Oriental population have less liver mitochondrial aldehyde dehydrogenase (ALDH2) activity than do other people. It was found that they possessed an enzyme with a lysine at position 487 (E487K) instead of glutamate (Glu487). We previously found that the \( K_m \) for NAD of recombinant human and rat E487K enzymes increased more than 150-fold (Farrés, J., Wang X., Takahashi, K., Cunningham, S. J., Wang, T.T., and Weiner, H (1994) \* J. Biol. Chem. 269, 13854–13860). Many aldehyde dehydrogenase-deficient people were found to be heterozygous when genotyped for ALDH2. In this study liver tissue from heterozygous people was analyzed and found to possess mRNAs for both the glutamate and the lysine subunits. Western blot analysis showed that the glutamate subunit was present. The cDNAs for Glu487 and E487K were coexpressed on one plasmid in \* Escherichia coli, and the enzyme forms were separated from each other by isoelectric focusing to show that heterotetramers were formed. One of the enzymes that cross-reacted with antibodies prepared against the active enzyme. Amino acid sequencing revealed that this variant differed from the active form by just one amino acid substitution. The Oriental variant possessed a lysine residue at position 487, while the active form contained a glutamate (6, 7). The accumulation of toxic acetaldehyde occurs after people with this phenotype drink ethanol. This leads to alcohol-associated symptoms, such as facial flushing and nausea (8, 9). It was unexpected to have found that the people possessing the inactive lysine-variant actually possessed the DNA coding for both the active glutamate enzyme and the inactive lysine enzyme (10, 11).

Three genotypes of ALDH2 have been identified in Oriental populations: \( \text{ALDH2}^{*1/\text{ALDH2}^{*1}} \), \( \text{ALDH2}^{*1/\text{ALDH2}^{*2}} \), and \( \text{ALDH2}^{*2/\text{ALDH2}^{*2}} \). The catalytic properties of the homotetramers of Glu487 and E487K have been studied. It was found that the Glu487 form had a low \( K_m \) for NAD, 30–70 \( \mu \text{M} \) (14, 15). The \( K_m \) value for NAD for the recombinantly expressed E487K increased over 150-fold (15). Although no detectable ALDH2 activity was found on electrophoregrams and in liver homogenates of ALDH2-deficient people (5, 16), the recombinantly expressed E487K was found to be active. The \( k_{\text{cat}} \) value was decreased 10- and 2.5-fold compared to that of the recombinant Glu487 human and rat forms (15), respectively.

It can be predicted that heterozygous people (\( \text{ALDH2}^{*1/\text{ALDH2}^{*2}} \)) should have 50% activity of the homozygous people (\( \text{ALDH2}^{*1/\text{ALDH2}^{*1}} \)) if inheritance of ALDH2 were co-dominant. Studies on the activity of ALDH2 in heterozygous individuals are conflicting. Activity staining of different tissue homogenates, after separation by IEF, showed the lack of ALDH2 activity in heterozygous people even though immunoreactions showed the presence of ALDH2 protein (11, 17–19). Other studies showed that heterozygous individuals had 13–15% of the Glu487 activity (16, 20). A lower percentage of people who were heterozygous for \( \text{ALDH2} \) had alcohol-associated symptoms after drinking ethanol than did people who were homozygous for \( \text{E487K} \) (21). This implies that the heterotetrameric ALDH2 might be catalytically active. None of these studies, however, determined whether or not both the E- and the K-subunits of ALDH2 were present in the heterozygous individuals. In a recent study it was reported that the insertion of the cDNA coding for the E487K subunit into a cell line expressing Glu487 subunits caused a reduction of the ALDH2 activity (22). The best interpretation of the latter observation is that the Glu487 subunits in the heterotetramers from the doubly transformed cell line had less activity than did the native enzyme.

In this study we investigate the reasons for finding less ALDH2 activity in the livers of people who were found to be
heterozygous with respect to the ALDH2 gene. We will demonstrate that mRNAs for both Glu<sup>487</sup> and E487K, as well as E-subunits of ALDH2, were present in heterozygous livers. The cDNAs for Glu<sup>487</sup> and E487K were coexpressed in <i>E. coli</i> to produce heterotetrameric forms of ALDH2 and the kinetic properties were determined. The data can best be interpreted as showing that the heterotetrameric form of ALDH2 had less activity than would be expected from the simple combination of subunits.

**EXPERIMENTAL PROCEDURES**

**Materials**—NAD and NADH were purchased from Sigma; Sequenase version 2.0 kit was obtained from U. S. Biochemical Corp.; propionaldehyde was from Aldrich; Magic Miniprep DNA purification system, random primers and T4 DNA ligase were from Promega Corp.; Eco<sub>57I</sub>, BamHI, and NdeI were from New England Biolabs; IEF standards and Affi-Gel 15 Bio-Gel agarose were from Bio-Rad; agarose gel and Pharmalyte were from Pharmacia Biotech Inc.; superscript reverse transcriptase II was from Life Technologies, Inc.; Taq DNA polymerase was from Boehringer Mannheim.

**RT-PCR**—Total RNA (5 µg) isolated from a heterozygous liver genotyped by David Crabb, M.D., Indiana University School of Medicine, was used for the synthesis of the first strand of cDNA by following the protocol from Life Technologies, Inc. Random primers were used for the reverse transcription which was performed at 42 °C for 1 h. The first strand of cDNA was then used as the template for PCR. The two primers, 5′-TTTGAATTCCATATGATGTGTTTGGAGCCCAGT-3′ (containing NdeI site, underligned) and 5′-TTTTGATCTTTATGAGTATCCT-3′ (containing BamHI site, underligned) were used to amplify the first 45 amino acids of human liver ALDH2. PCR was carried out for 30 cycles on a GeneAmp PCR System 9600 (PerkinElmer) using Taq DNA polymerase at 94 °C for 1 min, 49 °C for 2 min, and 72 °C for 2 min.

The RT-PCR products were digested with BamHI and NdeI and cloned into pT7-7 vector. The amplified cDNA was sequenced using the Sequenase version 2.0 kit. In addition, the RT-PCR products were digested with Eco<sub>57I</sub> for 2 h at 37 °C and then separated on a 2% agarose gel.

**Plasmid and Bacterial Strains**—The full-length cDNAs for human native and E487K ALDH2 (15, 23) were cloned on the pT7-7 vector. Each cDNA had its own ribosome binding site, but both were under the control of one T7 promoter. <i>E. coli DH5α</i> (Bio-Rad) was transformed with pT7-7 expression vector, and the constructions were confirmed by DNA sequencing. The T7 ALDH2 were expressed by the oncoexpression vector which was identical to that of the human coexpression vector, except the native and E487K were under control of separate T7 promoters. Restriction analysis using different enzymes was carried out to verify the presence of rat E- and K-cDNAs on the same plasmid. Human ALDH2 coexpression was performed in <i>E. coli</i> strain JM109(DE3) (Promega), and rat coexpression was in <i>E. coli</i> strain BL21(DE3) PLYsS.

**Expression of Heterotetramer ALDH2**—<i>E. coli</i> strain harboring the human coexpression vector pT7-7 were grown at 16 °C overnight after induction with 0.4 mM isopropyl-1-thio-β-D-galactopyranoside. The formation of NADH activity assays were performed with a Pharmacia flat-bed electrophoresis apparatus using Pharmalyte at 10 °C. Phosphorlytes, pH 4.5–5.4 and 4.0–6.5, were used for the human and rat samples, respectively. The gel was stained either for activity or for protein with Coomassie Blue (26). The concentrations of NAD and propionaldehyde were 5 mM and 100 mM, respectively, in the activity staining solution prepared in 100 mM sodium phosphate (pH 7.4).

**Identification of the mRNA for Glu<sup>487</sup> in the Heterozygous Liver**—To determine if mRNA for the Glu<sup>487</sup> variant was present in the liver of an ALDH2 heterozygous person, total liver RNA was analyzed by RT-PCR. The Oriental variant of ALDH2 differed from the active Glu<sup>487</sup> only at that position, so the cDNA encoding the last 45 amino acids of ALDH2, including position 487 was amplified to produce a 158-base pair fragment.

The 158-base pair RT-PCR product was treated with Eco<sub>57I</sub> to determine if mRNAs for both Glu<sup>487</sup> and E487K forms of ALDH2 were present in the heterozygous liver, as outlined by Tu and Israel (29). This enzyme recognizes the sequence 5′-CTGAAG(N)<sub>1–3</sub>3′ and 3′-GACCTCG(N)<sub>1–3</sub>-5′, which is present in the cDNA for the Glu<sup>487</sup> enzyme flanking the codons for position 487. When glutamate (GAA, underlined) at the position of 487 is replaced by lysine (AAA) the cDNA cannot be recognized by Eco<sub>57I</sub>. If mRNA for both native and E487K were present in the heterozygous liver, some of the RT-PCR products should be digested by Eco<sub>57I</sub>, others should not. As a control, the PCR products amplified from two pT7-7 plasmids (15), which carried the cDNAs encoding Glu<sup>487</sup> and E487K, respectively, were treated with Eco<sub>57I</sub>. The PCR products amplified from the cDNA encoding native form of human liver ALDH2 were digested as expected, while the PCR products of E487K were not cleaved by Eco<sub>57I</sub>. About half of the RT-PCR products amplified from the heterozygous liver were cleaved by the endonuclease, as shown in Fig. 1. The results of the Eco<sub>57I</sub> digestion indicated that the mRNAs for both Glu<sup>487</sup> and E487K were present in the heterozygous liver.

To further confirm the presence of mRNAs for Glu<sup>487</sup> and E487K in the heterozygous liver, RT-PCR products were cloned into the NdeI and BamHI sites of pT7-7 vector. Six colonies were sequenced; three were found to possess Glu<sup>487</sup> variant forms of ALDH2, having GAA at the position corresponding to 487. The other three possessed E487K, having AAA at this position. The sequencing results confirmed that the heterozygous liver had the mRNAs for both Glu<sup>487</sup> and E487K ALDH2.
Antibodies Established the Presence of Glu$^{487}$ Subunits in Livers from Heterozygous People—The antibodies prepared against the two synthetic peptides were used in cross-reaction experiments. Both antisera were found to cross-react with pure Glu$^{487}$ and E487K. An attempt was made to purify the antibodies so they would become determinant specific. Purified anti-E antibody recognized the native enzyme but not the E487K form. Unfortunately, purified antibodies prepared against E487K recognized both forms of ALDH2. It was possible, though, to use the anti-E antibody to determine if the E-subunits could be produced in the livers of heterozygous persons.

Human livers from people with different ALDH2 genotypes were analyzed by Western blotting as shown in Fig. 2. It was found that anti-ALDH2 (15) reacted equally well with liver homogenates from the homozygous E, the homozygous K, and heterozygous EK persons. Anti-E antibodies reacted with both homozygous E and heterozygous EK, but not with homozygous K liver homogenates. Neither anti-ALDH2 nor anti-E cross-reacted with cytoplasmic aldehyde dehydrogenase. These results indicated that the E-subunits were present in the heterozygous people. Due to the age of the liver samples and the potential differential stability of the subunits, it was not possible to accurately quantify the concentration of E- and K-subunits.

Expression and Purification of Coexpressed Human ALDH2—It appears that livers from people genotyped to be heterozygous contained both Glu$^{487}$ and E487K subunits. Assuming that the two subunits were equally expressed, one could expect that the tissue would possess 50% of the ALDH2 activity of the homozygous Glu$^{487}$ liver. However, the results were found to be different (11, 16–17, 20). It appears, supported by recent cell culture work (22), that the heterotetrameric enzyme had activities more like the E487K homotetramer had activities essentially identical to that of the native enzyme (15) and was so high and its specific activity so low, the contribution of the K-subunit to the overall activity would be small. To determine if it would even be possible to find a high $K_m$ component in the heterotetrameric mixture, equal concentrations of Glu$^{487}$ and E487K homotetramers were mixed and assayed. At NAD concentrations greater than 5 mM the Glu$^{487}$ enzyme was inhibited. If the E-subunits in the heterotetramer were subjected to NAD inhibition, it would not be possible to measure the activity of a high $K_m$ component. In fact, when the heterotetrameric enzyme was assayed above 5 mM NAD the velocity started to decrease. Thus, although we could not determine
that a high $K_m$ component was present, we cannot unequivocally state that one did not exist.

To show that it would be possible to assay for a high $K_m$ component, the rat enzymes were used. This was done because rat E487K possess a higher specific activity and a lower $K_m$ for NAD than human E487K (15). Both rat Glu$^{487}$ and E487K were mixed together in a ratio such that they possessed equal activity when assayed separately. A biphasic Lineweaver-Burke double reciprocal plot was obtained and the $K_m$ values were similar to ones found for the rat Glu$^{487}$ and E487K enzymes.

Pre-steady state burst analyses were performed for we previously showed that a burst of NADH formation existed with the human (26) and rat (15) native enzyme but not with the rat lysine variant (15). Due to the low yield of the human enzyme after IEF fractionation, it was not possible to determine the burst magnitude of the fractionated enzyme. Therefore, pre-steady state burst analysis was performed with enzymes only purified through 4'-hydroxyacetophenone-affinity chromatography. A burst magnitude of 2 was found with the Glu$^{487}$ homotetramers and no burst was found for E487K. Only a burst magnitude of 0.12 was found with the coexpressed heterotetramer enzyme. The fact that there was essentially no pre-steady state burst of NADH formation showed that the E-subunit in the heterotetramers was not functioning as it did in the homotetramers. Furthermore, the fact that the specific activity was just 16–18% compared to the homotetrameric Glu$^{487}$ form, allows us to suggest that the presence of E487K subunits alters the activity of the Glu$^{487}$ subunits, as was proposed to occur in the cell culture experiment (22).

Expression and Purification of Coexpressed Rat Liver Aldehyde Dehydrogenases—In a manner analogous to what was done with the human liver enzymes, cDNAs coding for the rat liver native and the E487K mutant were placed on one plasmid (Fig. 3). All three forms, homotetramer E, homotetramer K, and heterotetramer EK, were found to be expressed to the same extent. The enzymes were purified to homogeneity using the procedures previously described (15). IEF analysis showed, as was found with the human system, that the enzyme obtained from the coexpressed system had a pI value between those of the E- and K-homotetrameric enzymes (Fig. 4). The determinant-specific antibody, specific for the glutamate subunit, showed that the E-subunit was present in the coexpressed enzyme (data not shown).

Kinetic Properties of the Coexpressed Rat Aldehyde Dehydrogenases—We previously reported the kinetic constants for the recombinantly expressed rat liver enzyme forms (15). These were redetermined along with the one for the coexpressed heterotetrameric mixture and are presented in Table II. If the three enzyme forms were assayed with concentrations of NAD which would saturate only the low $K_m$ native enzyme, then the K-homotetrameric form had 12% of the activity of the E-homotetramer, while the heterotetramer had 34% of the activity of the coexpressed enzyme. The fact that there was essentially no pre-steady state burst of NADH formation showed that the E-subunit in the heterotetramers was not functioning as it did in the homotetramers. Furthermore, the fact that the specific activity was just 16–18% compared to the homotetrameric Glu$^{487}$ form, allows us to suggest that the presence of E487K subunits alters the activity of the Glu$^{487}$ subunits, as was proposed to occur in the cell culture experiment (22).
The activities are presented as nanomoles/min/mg of ALDH2. E487K enzymes measured at 0.8, 8, and 10 mM of NAD, respectively.

The assays were performed in 100 mM sodium phosphate, pH 7.4, with 28 µM propionaldehyde. The activities are represented as nanomoles/min/mg of ALDH2. The lower numbers were obtained from the nonlinear regression analysis, while the upper values were from the linear regression analysis. The analyses were performed using the MicroMath Scientist Statistics program.

TABLE I
Kinetic properties of human Glu487, E487K, and heterotetrameric ALDH2s

| Enzyme  | $K_m$ for NAD (µM) | $V_{max}$ (µM) | Activity % |
|---------|-------------------|----------------|------------|
| Glu487  | 31 ± 2            | 963 ± 40       | 100        |
|         | 36 ± 4            | 1030 ± 35      | 100        |
| E487K   | 6500 ± 770        | 82 ± 9         | 8.5        |
|         | 7300 ± 1000       | 81 ± 7         | 7.9        |
| Heterotramer | 117 ± 6    | 175 ± 7        | 18         |
|         | 91 ± 6            | 162 ± 2        | 16         |

TABLE II
Kinetic properties of rat Glu487, E487K, and coexpressed ALDH2s

| Enzyme  | $V_{max}$ (µM) | % Activity | Activity (0.8 mM NAD) |
|---------|----------------|------------|-----------------------|
| Glu487  | 552            | 100        | 552                   |
|         | 204            | 37         | 66                    |
| Coexpressed | 285       | 48         | 188                   |

have expected that the heterozygous persons have had approximately 50% of the activity of a person who was homozygous with respect to the ALDH2*1 gene. One explanation for not finding the ALDH2 activity in these livers is that Glu487 subunits were not expressed due to transcriptional or translational alterations. An alternative explanation is that the heterotetrameric enzyme behaves more like a homotetramer of the K-subunits. Evidence recently has been presented to show that, in cells grown in culture, the addition of the lysine-coding ALDH2 cDNA caused a suppression of the activity of the glutamate enzyme (22). This was the first evidence to suggest that the E-subunit in the heterotetrameric enzyme was less active.

To test for these possibilities we examined liver samples from people who were previously genotyped for ALDH2. RT-PCR analysis showed that two cDNA sequences were obtained from the liver of the heterozygous individual, one with the lysine codon (AAA), other with the glutamate codon (GAA). This demonstrated that the mRNA for each form of the enzyme was transcribed in the liver of the heterozygous person. However, the existence of the mRNA does not prove that protein is produced. Instead of sequencing the entire cDNA to determine if there were a premature stop codon or an alteration which could affect translation, an antibody approach was employed. Antibodies were prepared against the C-terminal 16 amino acids of the Glu487 and E487K enzymes. Liver homogenates from the two heterozygous people analyzed were found to possess the glutamate subunits, which could not be detected in two liver homogenates from people who were genotyped to be homozygous for the ALDH2*2. This supports the notion that the liver of a heterozygous person had the glutamate subunit of ALDH2.

It was necessary to measure the activity of the heterotetrameric forms of human liver ALDH2 composed of both E- and K-subunits to determine why the activity in the heterozygous person was lower than what was expected if the two forms of ALDH2 were co-dominantly inherited. Since we did not have access to large quantities of fresh human liver tissue from genotyped people, we reverted to using an E. coli coexpression system. The cDNA for both the lysine and the glutamate subunits were cloned on one plasmid. The coexpressed enzymes after IEF separation were found between the pI values for the homotetrameric Glu487 and E487K enzymes. Similar results were found with the rat coexpressed enzymes.

The purified human enzyme was fractionated by preparative IEF. The fractions of active enzyme were selected which had pI values falling between those of the two pure homotetramers. Thus, we could be assured that the activities being measured was not due to the presence of homotetramers of the E- or the K-subunits. From the pure heterotetramer only one low $K_m$ for NAD was found and the specific activity was 16–18% of Glu487 homotetramer. When the specific activity was measured at 2.5 mM NAD, a concentration which would saturate the E subunit, the heterotetramer possessed only 13% activity. Under these assay conditions, E487K homotetramer had a specific activity of just 2.5%. Even though the precise subunit compositions of these heterotetramers were unknown, we conclude that heterotetramers were less active and possessed kinetic properties different from the parent homotetramers.

Assuming that there were an equal number of E- and K-subunits expressed, then independently of how they were assembled, one would have expected the heterotetramers to have 50% the specific activity of the Glu487 homotetramer when assayed at the low concentration of NAD which would saturate only the E-subunits. Under these conditions, though, only 13% of the activity was found, indicating that the presence of the K-subunit did indeed suppress the activity of the E-subunit, supporting the conclusions from the cell culture work (22).

We previously showed that the human Glu487 enzyme had a pre-steady state burst of approximately 2 moles of NADH per mole of enzyme (26), indicative of the half-of-the-site reactivity. No burst was found with the human E487K enzyme as we previously showed for the rat E487K enzyme (15). The human coexpressed enzyme had a burst magnitude of only 0.12, not a value of 1 or 2 as would be expected if the E-subunits were acting independent of the K-subunits. The 0.12 burst magnitude might be due to the presence of a small concentration of
homotetrameric Glu\textsuperscript{487} in the coexpressed system because the coexpressed ALDH2 used for the burst assay was not fractionated by IEF. The rate-limiting step changed for the E-subunit in the heterotetramer. It appears, then, that the E-subunit was not acting independently of the K-subunit and was not functioning as it would in the homotetrameric glutamate enzyme.

It is difficult to predict exactly what properties could be expected to be found for a heterotetramer composed of both high active and low active subunits. First, as discussed, the K-subunit decreases the activity of the E-subunit, but the exact amount is not known. The second complexity is that ALDH2 functions with half-of-the-site reactivity (26, 27). That is, in the tetramer the subunits are not acting independently of each other but are functioning as two pairs and not four independent subunits. Assuming that an equal concentration of the E- and the K-subunits were expressed and that assembly were truly random, then the distribution of species should have been as illustrated in Scheme 1. Since the heterotetramers were removed, the percentage of the heterotetramers would have been 28.7, 42.5, and 28.7 for the E\_E K\_K, E\_K K\_E, and E\_K K\_K, respectively. In any tetramer, six pairs of dimers could theoretically exist in the total population, as is illustrated in Table III. If we define the total activity of the Glu\textsuperscript{487} homotetramer as 100, then each of six E\_E dimer pairs in the half-of-the-site model would have an activity of 16.6. When assayed at 2.5 mM NAD the activity of the E487K homotetramer was just 2.5% that of the Glu\textsuperscript{487} homotetramer. Thus, each of the six K\_K dimer pairs would have an activity of 0.4. The expected activity for each heterotetrameric species is tabulated in Table III, assuming that the E\_E dimer pair had the activity of the KK-homodimer. In K\_E, where there are three possible pairs of E\_E interactions in the half-of-the-site model, the enzyme would have 51.2% the activity of the E\_E-homotetramer. In contrast, if the subunits were functioning independent of each other, the species would have had 75.6% the activity of the E\_E homotetramer. Similarly, the E\_K\_K\_K form would have just 18.7% the activity in the half-of-the-site model compared to 51.2% in the full-site model. Lastly, E\_K\_K\_K would have had essential no activity in the half-of-the-site model compared to 26.8% in the full-site model. On average, the heterotetramer mixture would have had approximately 51.1% the activity of the E\_K homotetramer if the subunits were truly independent of each other, but only 23.3% if the subunits were functioning as a pair of dimers and the EK pair had properties similar to a KK pair. We found that the heterotetramer had just 13% the activity of the high active homotetramer. This shows that the K-subunit decreases the activity of the E-subunit, as was suggested by the cell culture experiments (22).

We cannot offer an explanation for how the K-subunits alters the properties of the E-subunit. It appears to be a result of a change in rate-limiting step as the E-subunit no longer had a pre-steady state burst. Other investigators have reported that hetero-systems did not function as the sum of the properties of the independent subunits. This has been shown to occur with lactate dehydrogenase (31) and with aspartate transcarbamylase (32). The three dimensional structure of ALDH2 is not known so it is not possible to discuss subunit interactions. It is possible, though, that a glutamate residue in one subunit is interacting with a lysine in another in the heterotetrameric form. This new salt bond could be responsible for the altered properties of the heterodimer. Furthermore, if there were an attraction for heterotetrameric formation, the distribution of dimer pairs would not be the statistical average as illustrated in Table III but, would be skewed toward more E\_K\_K heterotetramers. This condition could exist because the activity of the mixture was lower than the value estimated from the statistical model.

We reported previously that the recombinantly expressed rat E487K homotetramer had a high $K_a$ for NAD but, the specific activity was about 40% of the rat Glu\textsuperscript{487} enzyme (15). This was reconfirmed in this study. Analysis of the kinetic data at a low concentration of NAD showed that E487K homotetramer had 12% and the heterotetramers had 34% the activity of the native enzyme. In contrast, the human E487K homotetramer had just 2.5% and heterotetramers had 13% the native human enzyme activity. Thus, it appears that the K-subunit in the human enzyme more dramatically affects the activity of the E-subunit than it does in the rat liver enzyme.

The amino acid sequences of the rat and human mitochondrial ALDH2 are 95% identical (30). Hence, it is difficult to rationalize why the change of a glutamate to a lysine at position 487 would produce such different affects on the specific activity of the rat and human enzymes. Investigators have discussed inserting the K-cDNA into animals to determine if this will affect their ethanol drinking behavior. Based on the data obtained in this study, we can conclude that in a liver of a transgenic rodent, the heterotetrameric ALDH2 might be much more active than it is in human heterozygous liver. The aldehyde oxidizing capacity in the rat liver will be larger than what would be expected to be found in heterozygous human. How this will affect acetaldehyde metabolism is not known. It is the increased blood acetaldehyde concentration that has been postulated to be the deterrent for alcohol drinking in Oriental people who are heterozygous with respect to their ALDH2 (16, 21). Thus, the transgenic animal may not be a good model to study ethanol toxicity found in heterozygous Oriental people.
REFERENCES

1. Svanas, G. W., and Weiner, H. (1985) Arch. Biochem. Biophys. 236, 36–46
2. Cao, Q. N., Tu, G.-C., and Weiner, H. (1988) Alcohol. Clin. Exp. Res. 12, 720–724
3. Teng, Y.-S. (1981) Biochem. Genet. 19, 107–114
4. Impraim, C., Wang, G., and Yoshida, A. (1982) Am. J. Hum. Genet. 34, 837–841
5. Ikawa, M., Impraim, C. C., Wang, G., and Yoshida, A. (1988) J. Biol. Chem. 253, 6282–6287
6. Yoshida, A., Huang, I.-Y., and Ikawa, M. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 258–261
7. Hempel, J., Kaiser, R., and Jornvall, H. (1984) FEBS Lett. 172, 367–373
8. Mizoi, Y., Ijiri, I., Tatsuno, Y., Kijima, T., Fujiwara, S., and Adachi, J. (1979) Pharmacol. Biochem. Behav. 10, 303–311
9. Harada, S., Agarwal, D. P., and Goedde, H. W. (1981) Lancet 2, 982
10. Crabb, D. W., Edenberg, H. J., Bosron, W. F., and Li, T.-K. (1989) J. Clin. Invest. 83, 314–316
11. Goedde, H. W., Singh, S., Agarwal, D. P., Fritzke, G., Stapel, K., and Paik, Y. K. (1989) Hum. Genet. 81, 305–307
12. Yoshida, A., Wang, G., and Dave, V. (1983) Am. J. Hum. Genet. 35, 1107–1116
13. Hsu, L. C., Bendel, R. E., and Yoshida, A. (1987) Am. J. Hum. Genet. 41, 996–1001
14. Greenfield, N. J., and Pietruszko, R. (1977) Biochim. Biophys. Acta 483, 35–45
15. Farrés, J., Wang, X., Takahashi, K., Cunningham, S. J., Wang, T. T., and Weiner, H. (1984) J. Biol. Chem. 269, 13854–13860
16. Enomoto, N., Takase, S., Yasuhara, M., and Takada, A. (1991) Alcohol. Clin. Exp. Res. 15, 141–144
17. Goedde, H. W., Agarwal, D. P., Eckey, R., and Harada, S. (1985) Alcohol 2, 383–390
18. Chao, Y. C., Liu, S. R., Tsai, S. F., and Yin, S. J. (1986) Proc. Natl. Sci. Counc. Repub. China B 17, 98–102
19. Yamamoto, K., Ueno, Y., Mizoi, Y., and Tatsuno, Y. (1993) Arukoru Kenkyu. To Yakubutsu Izon 28, 13–25
20. Ferencz-Biro, K., and Pietruszko, R. (1984) Biochem. Biophys. Res. Commun. 118, 97–102
21. Takeda, T., Morimoto, K., Mao, X., Hashimoto, T., and Furuyama, J. (1994) Hum. Genet. 94, 217–223
22. Xiao, Q., Weiner, H., Johnston, T., and Crabb, D. W. (1995) J. Clin. Invest. 96, 2180–2186
23. Zheng, C.-F., Wang, T. T., and Weiner, H. (1992) Alcohol. Clin. Exp. Res. 17, 828–831
24. Ghenbot, G., and Weiner, H. (1992) Protein Expr. Purif. 3, 470–478
25. Takahashi, K., Weiner, H., and Hu, J. H. (1980) Arch. Biochem. Biophys. 205, 571–578
26. Wang, X., and Weiner, H. (1995) Biochemistry 34, 237–243
27. Weiner, H., Hu, J. H., and Sanny, C. G. (1976) J. Biol. Chem. 251, 3853–3855
28. Tam, J. P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5409–5413
29. Tu, G.-C., and Israel, Y. (1993) Eur. J. Clin. Chem. Clin. Biochem. 31, 591–594
30. Hempel, J., Nicholos, H., and Lindahl, R. (1993) Protein Sci. 2, 1980–1990
31. Ainslie, G. R. and Cleland, W. W. (1982) Arch. Biochem. Biophys. 216, 101–104
32. Eisenstein, E., Han, M. S. Woo, T. S., Ritchey, J. M., Gibbons, I., Yang, Y. R., and Schachman, H. K. (1992) J. Biol. Chem. 267, 22148–22155