Proc. Natl. Acad. Sci. USA
Vol. 77, No. 10, pp. 5784–5788, October 1980
Biochemistry

New molecular forms of human liver alcohol dehydrogenase: Isolation and characterization of $ADH_{Indianapolis}$

(alcohol:NAD^+ oxidoreductase/alcohol metabolism/isoenzymes)

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ABSTRACT **The biochemical determinants of alcoholism and genetic correlates for the variability in man's response to alcohol have remained obscure until recently. The identification of genetically determined isoenzymes of alcohol dehydrogenase with different catalytic properties may bear importantly upon this problem. New molecular forms of human liver alcohol dehydrogenase (ADH; alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) have recently been identified in 16% of the liver specimens from an urban population from Indianapolis, Indiana [Bosron, W. F., Li, T.-K. & Vallee, B. L. (1979) *Biochem. Biophys. Res. Commun.* 91, 1549–1555]. The distinguishing features of these specimens were (*i*) they showed activity optima for ethanol oxidation at both pH 7.0 and 10.0 and (*ii*) they formed electrophoretic bands cathodic to the $\beta\beta$ isoenzyme. From such livers, three new ADH forms have now been isolated, one of which has a single pH optimum at 7.0 and two of which have dual optima at pH 7.0 and 10.0. These new forms were designated $ADH_{Indianapolis}$ forms 1, 2, and 3, respectively. They can be differentiated from previously described ADH isoenzymes, including the so-called "atypical" isoenzyme, by their electrophoretic mobility, pH optima, and K_m for ethanol (approximately 60 mM at pH 7.5). Based upon the electrophoretic pattern of livers containing $ADH_{Indianapolis}$ and the mobility of the three isolated molecular forms, $ADH_{Indianapolis}$ may be the result of polymorphism at the ADH_2 gene locus, which codes for the β subunit.**

Humans exhibit large individual differences in their rate of alcohol metabolism and in their physiological, psychological, and pathological response to ethanol consumption (1). The potential relationships between these phenomena and the remarkable variability and large number of alcohol dehydrogenase (ADH; alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) isoenzymes in human liver have been discussed (2). Such comparisons are of quite recent origin largely because a means for the isolation and characterization of the molecular properties of human ADH isoenzymes had not been available. Specific affinity chromatographic techniques for ADH isoenzyme purification (3, 4) and electrophoretic procedures capable of resolving hitherto unidentified molecular forms (5, 6) have improved the situation rapidly and significantly.

A genetic model‡ proposed some years ago (7) has thus far satisfactorily accounted for most variations in ADH isoenzymes observed in earlier studies. It did not encompass π-ADH (2), however, which was discovered subsequent to the proposal of the model. Most recently, we discovered yet other ADH molecular forms in 16% of autopsy liver specimens from Indianapolis, IN (6), which also could not be classified according to that model. Characteristically these livers manifest two pH optima for ethanol oxidation at pH 7.0 and 10.0 and form electrophoretic bands cathodic to the $\beta\beta$ isoenzyme (6).

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We have now isolated three of these new molecular forms from such liver specimens. One of them exhibits a single activity optimum at pH 7.0, whereas the other two have dual pH optima at 7.0 and 10.0. Based on the pH-dependence of their activities, their K_m values for ethanol, and their mobility on starch gel electrophoresis, these newly discovered forms can be differentiated from both the "typical" and "atypical" enzyme forms described previously (5–9). Clearly, the molecular heterogeneity of human liver ADH is much more complex and diverse than had been appreciated, which calls for a more searching examination of the molecular and genetic basis of ADH isoenzymes.

This communication will use a system of nomenclature for the newly discovered ADH forms that is similar to that conventionally used for the hemoglobins. Those ADH forms exhibiting activity optima for ethanol oxidation at pH 7.0 are designated collectively as $ADH_{Indianapolis}$. Isoenzymes migrating cathodic to $\beta\beta$ but exhibiting a pH-optimum for ethanol oxidation at 8.5 rather than at 10.0 and initially thought to be uniquely "atypical" (8, 9) might more appropriately be called ADH_{Bern}, because they were first recognized in a Swiss population. ADH forms similar to ADH_{Bern} are also present in 85% or more of Asian populations (10–12). Because the chemical identities of these forms and ADH_{Bern} have not been established, the "atypical" ADH in liver specimens of Asian donors from Honolulu, Hawaii used in this study will be referred to as $ADH_{Honolulu}$.

METHODS

Livers from apparently healthy adults who had succumbed suddenly, were obtained within 12 hr postmortem and stored at −55°C. Autopsy specimens were obtained from the Departments of Pathology, Wishard Memorial Hospital, Indianapolis, IN and St. Francis Hospital, Honolulu, HA. Informed consent was obtained with assurance to protect the anonymity of the donor. ADH activity and electrophoretic properties were determined on 2–5 g of liver, homogenized in 2–5 ml of 50 mM sodium phosphate (pH 7.5) at 4°C with a Brinkman Polytron. Homogenates were centrifuged for 60 min at 100,000 × *g*.

ADH activity was determined spectrophotometrically at 340 nm by monitoring the production of NADH after exhaustion

Abbreviation: ADH, alcohol dehydrogenase.

‡ The genetic model for formation of ADH molecular forms as isoenzymes assumes three separate gene loci, ADH_1, ADH_2, and ADH_3, coding for subunit polypeptide chains α, β, and γ, respectively. It is further proposed that genetic polymorphism occurs at the ADH_3 locus with two different alleles, ADH_3^1 and ADH_3^2, giving rise to γ_1 and γ_2 subunits, respectively. The combination of these subunits into active dimeric enzyme yields an array of homodimeric $\alpha\alpha$, $\beta\beta$, $\gamma_1\gamma_1$, $\gamma_2\gamma_2$ and heterodimeric $\alpha\beta$, $\alpha\gamma_1$, $\alpha\gamma_2$, $\beta\gamma_1$, $\beta\gamma_2$ and $\gamma_1\gamma_2$ isoenzymes as identified by starch gel electrophoresis in Figs. 2 and 4.

of the blank reaction owing to endogenous substrates in the homogenate supernatant (6). Protein concentration was determined with the biuret reagent (13) for homogenate supernatants and by the method of Lowry *et al.* (14) for purified ADH forms. Specific activities were expressed as μmol/min (units) per mg of cytosolic protein. Starch gel electrophoresis and staining of gels for ADH activity has been described (6). ADH molecular forms were purified by chromatography on DEAE-cellulose, 4[3-(*N*-6-aminocaproyl)aminopropyl]pyrazole-Sepharose, and CM-cellulose (3, 4).

RESULTS

Electrophoretic and Catalytic Properties of Liver Homogenates Containing $ADH_{Indianapolis}$ and $ADH_{Honolulu}$. Seven out of eight liver specimens from donors of Asian origin collected in Honolulu had an optimum activity for ethanol oxidation at pH 8.5–9.0 (Fig. 1*B*). The enzyme forms with the pH 8.5 optimum for activity in these livers will be called $ADH_{Honolulu}$. By contrast, specimens containing $ADH_{Indianapolis}$ exhibited dual pH optima at approximately 7.0 and 10.0 (Fig. 1*A*), whereas those containing neither of these forms had a single pH optimum at 10.0 or greater (6).

Specimens containing $ADH_{Indianapolis}$ and those containing $ADH_{Honolulu}$ formed bands that migrated cathodic to the $\beta\beta$ isoenzyme in starch gel electrophoresis. However, they could be differentiated from each other and from specimens containing neither of these forms. Livers containing $ADH_{Honolulu}$ exhibited three broad, intense bands (Fig. 2, lane b) after staining for activity at pH 8.5. One of these characteristically migrated cathodic to the $\beta\beta$ isoenzyme. On the other hand, livers containing $ADH_{Indianapolis}$ exhibited two different starch gel electrophoresis patterns. That shown in Fig. 2, lane d, was observed in 14 out of 106 liver specimens. It contained several activity bands that could not be identified as combinants of the α, β, γ_1, and γ_2 subunits, according to the genetic model of Smith *et al.* (7). One of the bands was more cathodic than the

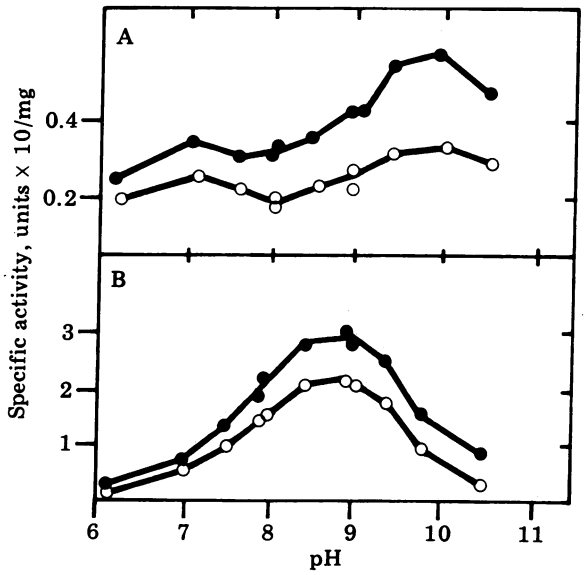

FIG. 1. pH–enzyme activity profiles of liver homogenate supernatants. ADH activity was determined with 33 mM ethanol and 2.4 mM NAD^+ at 25°C in 33 mM sodium phosphate, pH 6–8/33 mM sodium barbital, pH 8–9/33 mM glycine adjusted to pH 9–10.5 with NaOH. Two representative profiles for liver specimens containing $ADH_{Indianapolis}$ (*A*) and $ADH_{Honolulu}$ (*B*).

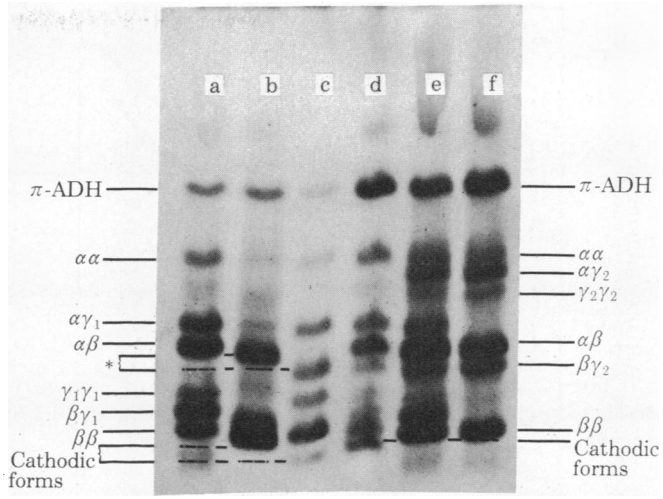

FIG. 2. Starch gel electrophoresis of human liver ADH. High-voltage starch gel electrophoresis was performed on liver homogenate supernatants (6). Lanes: a, phenotype ADH_3^1; e, phenotype $ADH_3^{2\text{-}1}$; f, phenotype ADH_3^2 [phenotypes according to the model of Smith *et al.* (7)]; b, $ADH_{Honolulu}$; c and d, $ADH_{Indianapolis}$. Lanes b, c, and d contain bands migrating cathodic to the $\beta\beta$ isoenzyme and unidentifiable bands (*) with mobility intermediate between $\alpha\beta$ and $\beta\beta$.

$\beta\beta$ isoenzyme. The electrophoretic pattern shown in Fig. 2, lane c, was observed in 2 of 106 liver specimens from Indianapolis. Whereas π-ADH and the $\alpha\alpha$, $\alpha\gamma_1$, and $\gamma_1\gamma_1$ isoenzymes usually found in livers with the ADH_3^1 phenotype (Fig. 2, lane a) were present, other forms characteristic of this phenotype—$\alpha\beta$, $\beta\gamma_1$, and $\beta\beta$—were absent. They appeared to be replaced by three new bands, each exhibiting the same degree of increased mobility toward the cathode relative to the $\alpha\beta$, $\beta\gamma_1$, and $\beta\beta$ isoenzymes, respectively.

To further establish criteria for differentiating liver specimens that contain $ADH_{Indianapolis}$ from those containing $ADH_{Honolulu}$, we measured the ethanol concentration-dependence of ADH activity in liver homogenate supernatants in the presence and absence of 0.2 mM 4-methylpyrazole in order to eliminate the contribution of π-ADH (2). As shown in Fig. 3*B*, the 4-methylpyrazole-inhibitable activity of specimens containing $ADH_{Honolulu}$ became maximal at approximately 15 mM ethanol, and substrate inhibition occurred above this concentration. However, ethanol-oxidizing activity for liver homogenates containing $ADH_{Indianapolis}$ did not approach saturation even at substrate concentrations of 100 mM (Fig. 3*A*). In contrast, activity in livers that contained neither $ADH_{Honolulu}$ nor $ADH_{Indianapolis}$ was maximal at 5 mM ethanol (2).

Isolation and Characterization of Different Molecular Forms of $ADH_{Indianapolis}$. The seven enzyme forms in a liver specimen exhibiting the same electrophoretic pattern as that shown in Fig. 2, lane c, were separated and purified in the following manner. Six of them were purified collectively from contaminating proteins by column chromatography 4[3-(*N*-6-aminocaproyl)aminopropyl]pyrazole-Sepharose and simultaneously separated from π-ADH (2, 4). They were then separated from each other by chromatography on CM-cellulose. Fig. 4 compares their electrophoretic mobilities with those of enzyme forms present both in the original liver homogenate supernatant (Fig. 4, lane a) and in a liver that had a single pH optimum at 10.0–10.5 and contained π-ADH, $\alpha\alpha$, $\alpha\gamma_1$, $\alpha\beta$, $\gamma_1\gamma_1$, $\beta\gamma_1$, and $\beta\beta$ (Fig. 4, lane b). Three of the purified forms could be identified as $\alpha\alpha$, $\alpha\gamma_1$, and $\gamma_1\gamma_1$. Each of these exhibited a single pH optimum at 10.5 (Fig. 5*A*). The apparent K_m values for ethanol determined in 0.1 M sodium phosphate

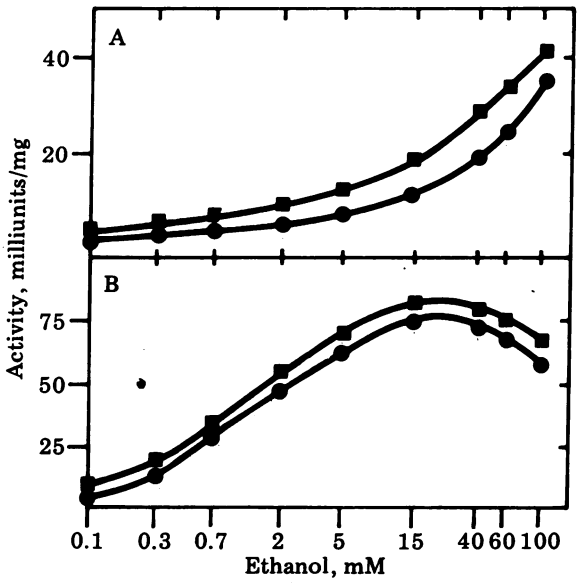

FIG. 3. Alcohol concentration dependence of ADH activity in homogenate supernatants. ADH activity was assayed in 0.1 M sodium phosphate (pH 7.5) at 25°C with 0.1–100 mM ethanol and 2.4 mM NAD^+. The contribution of π-ADH to the total activity was subtracted by plotting the activity obtained in the absence of 0.2 mM 4-methylpyrazole minus that obtained in its presence (2). Two representative profiles for livers containing $ADH_{Indianapolis}$ (*A*) and $ADH_{Honolulu}$ (*B*).

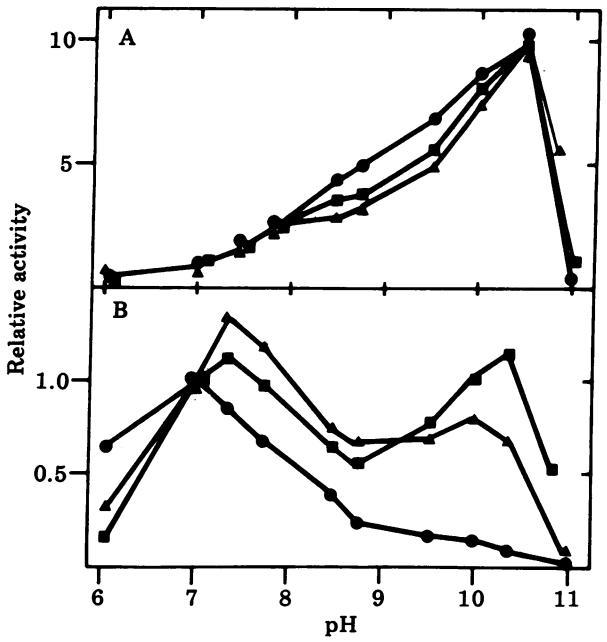

FIG. 5. pH–enzyme activity profiles of isolated ADH molecular forms. The pH-dependence of enzyme activity for the six isolated molecular forms described in Fig. 4 was determined as in Fig. 1. *A*, Profiles for the $\alpha\alpha$ (●—●), $\alpha\gamma_1$ (■—■), and $\gamma_1\gamma_1$ (▲—▲) isoenzymes. *B*, Profiles for $ADH_{Indianapolis}$ forms 1 (●—●), 2 (■—■), and 3 (▲—▲). ADH activity was normalized to that at pH 7.0.

(pH 7.5) with 2.4 mM NAD^+ were: $\alpha\alpha$, 5.3 mM; $\alpha\gamma_1$, 1.2 mM; $\gamma_1\gamma_1$, 0.31 mM. The remaining three forms had electrophoretic mobilities cathodic to those of $\beta\beta$, $\beta\gamma_1$, and $\alpha\beta$. We have designated them $ADH_{Indianapolis}$ forms 1, 2, and 3, respectively. $ADH_{Indianapolis}$ form 1 exhibited a single pH optimum at 7.0, whereas $ADH_{Indianapolis}$ forms 2 and 3 exhibited dual pH optima for activity at 7.0–7.5 and 10.0–10.5 (Fig. 5*B*). The apparent K_m values for ethanol of these three forms were high, ranging from 56–74 mM.

DISCUSSION

Alcohol abuse and alcohol-related pathology manifest largely as behavioral and sociological abnormalities. Most attempts at discerning cause–effect relationships have been directed toward these areas in which ill effects are most apparent. Such manifestations have been resistant to experimental study in other species—a major handicap to this pursuit. Conversely, biochemical efforts in humans have been hindered by the un-

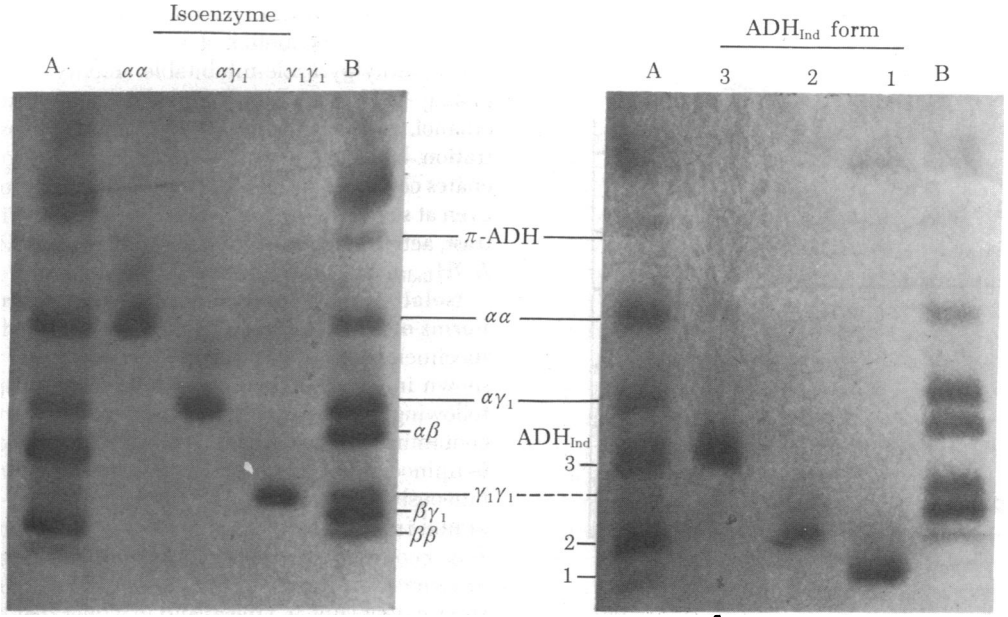

FIG. 4. Starch gel electrophoresis of isolated ADH molecular forms. Six of the molecular forms present in a liver that contained $ADH_{Indianapolis}$ and showed the starch gel electrophoretic pattern of Fig. 2, lane c, were isolated by column chromatography on 4[3-(*N*-6-aminocaproyl)aminopropyl]pyrazole-Sepharose and CM-cellulose. Lanes; a, pattern of the liver homogenate supernatant before fractionation; b, that of a liver classified as phenotype ADH_3^1 (Fig. 2, lane a). (*Left*) The electrophoretic mobility of three of the isolated forms indicated as the $\alpha\alpha$, $\alpha\gamma_1$, and $\gamma_1\gamma_1$ isoenzymes. (*Right*) $ADH_{Indianapolis}$ forms 1, 2, and 3. The broad bands appearing anodic to π-ADH (i.e., at the top of lanes in Figs. 2 and 4) are lactate dehydrogenase.

availability of purified liver ADH and, hence, the lack of knowledge regarding its physical, chemical, and enzymatic properties. Based on precedents in the evolution of other enzymes and proteins, it seemed reasonable to assume that human ADH would closely resemble that of other species. Hence, the role of ADH in regulating alcohol metabolism and alcoholism has been studied extensively in experimental animals, particularly in the rat (1, 15). Although such studies have yielded fundamental information, it has become increasingly apparent that degrees of complexity exist in the human enzyme that have no counterpart in lower animal species.

Previous efforts to discern the extent of genetic variation in human ADH isoenzymes have been hampered by the lack of suitable methods for isoenzyme separation. The improved resolution gained through the use of high-voltage starch gel electrophoresis has now enabled the definitive identification of the isoenzymes described by Smith *et al.*, (7) and of π-ADH by a single gel (5, 6). It has also facilitated the discovery of new enzyme forms—e.g., $ADH_{Indianapolis}$. The dual pH optima for enzyme activity at 7.0 and 10.0 and enzyme activity bands cathodic to $\beta\beta$ isoenzyme on starch gel electrophoresis (6) are the most readily recognized features of livers containing $ADH_{Indianapolis}$. As demonstrated in this study, the bimodal pH–enzyme activity profile of liver homogenate supernatants exhibiting the electrophoretic pattern in Fig. 2, lane c, reflects the contributions of three different groups of enzyme forms: two with a single pH optimum at either 10.0 or 7.0 and a third with pH optima at both 7.0 and 10.0. The enzyme activity band migrating cathodic to the $\beta\beta$ isoenzyme is the one with the single pH optimum at 7.0 (Figs. 4 and 5). The pH–enzyme activity profile of the cathodic band in livers with the electrophoretic pattern in Fig. 2, lane d has not yet been determined.

Liver specimens manifesting enzyme activity bands with electrophoretic mobility greater than that of $\beta\beta$ or showing "atypical" pH (10.5–8.5)–enzyme activity ratios [i.e., below 1.0 (9)] have been observed in the past with different degrees of frequency in different populations (5, 7, 9, 10, 12). In several studies, the homogenates of such livers have been shown to have a single optimum for ethanol oxidation at 8.5 (8, 11, 16). However, only one enzyme form with both of these properties, present in 20% of a Swiss population, has been isolated and characterized biochemically (8, 17, 18). This enzyme form, initially named "atypical" ADH, is designated ADH_{Bern}, according to the nomenclature used here. In agreement with previous reports (10–12), we find that more than 80% of the liver specimens from Asian donors form enzyme bands cathodic to $\beta\beta$ and have a pH optimum for enzyme activity at 8.5. Because it cannot be stated with certainty whether or not the chemical properties of these enzyme forms are identical to ADH_{Bern}, they are called $ADH_{Honolulu}$ in this study. Clearly, however, the livers containing these enzyme forms differ from those containing the newly discovered $ADH_{Indianapolis}$ not only in their pH–enzyme activity profiles (Fig. 1) and starch gel electrophoretic patterns when stained at pH 8.5 (Fig. 2) but also in their saturation kinetics with ethanol (Fig. 3). Preliminary studies of the catalytic properties of the three isolated $ADH_{Indianapolis}$ forms indicate that their apparent K_m values for ethanol range from 56 to 74 mM at pH 7.5—values that are substantially higher than those reported for ADH_{Bern} (3.1 mM), $\beta\beta$ [1.8 mM at pH 8.0 (19)], or π-ADH [34 mM at pH 7.5 (20)].

It has been postulated that ADH_{Bern} is the result of polymorphism at the ADH_2 locus that codes for the β subunit (7, 17). Moreover, Azevedo *et al.* (21) have suggested that the cathodic enzyme bands that appear in samples from about 1% of a Brazilian population (with unknown pH–enzyme activity profiles) represent yet another allele at the ADH_2 locus. Studies to validate the proposed subunit structure and genetic assignment of these cathodic forms—e.g., by dissociation and recombination experiments with isolated isoenzymes (22)—have yet to be performed. For example, it is not clear whether ADH_{Bern} is heterodimeric or a mixture of hetero- and homodimeric forms (18, 22). Nevertheless, by analogy to the preceding hypotheses concerning cathodic enzyme bands, $ADH_{Indianapolis}$ may be the product of yet another allele at the ADH_2 locus. The form with the most cathodic band, $ADH_{Indianapolis}$ form 1, which has a single optimum for activity at pH 7.0, could conceivably be the homodimeric isoenzyme of this newly discovered $\beta_{Indianapolis}$ subunit. The dual pH optima for activity at 7.0 and 10.0 and the electrophoretic mobilities of $ADH_{Indianapolis}$ forms 2 and 3 would be consistent with their being heterodimeric combinants of a $\beta_{Indianapolis}$ subunit with γ_1 and α subunits, respectively. Thus, the liver specimen showing the electrophoretic pattern in Fig. 1, lane c, would be homozygous for this allele at the ADH_2 locus. We are currently examining the hypothesis that livers represented by the sample in Fig. 1, lane d, are heterozygous for this allele at the ADH_2 locus. Alternatively, $ADH_{Indianapolis}$ could be the product of an entirely different gene locus. Hybridization experiments with the presumed hetero- and homodimeric forms and their chemical characterization are required to test this model of genetic polymorphism at the ADH_2 gene locus.

Since ADH is the principal and rate-limiting enzyme of ethanol metabolism, genetic variation in expression of ADH isoenzymes in human liver might be thought to underlie individual and racial differences in rates of alcohol metabolism (1, 2). In this regard, it is particularly interesting that in this study $ADH_{Honolulu}$ was found in seven out of eight specimens from donors of Asian origin but in only 1 of the 106 liver specimens from the racially mixed, urban population from Indianapolis. Moreover, we have observed that $ADH_{Indianapolis}$ was present in 16 of 57, or 28%, of the liver specimens from black Americans but in none of the 49 specimens from white Americans.

Most of the knowledge of the properties of liver ADH has been obtained from studies on purified isoenzyme forms of the horse and rat or on preparations of the human enzyme containing mixtures of enzyme forms (1). Although initial investigations of the human enzyme did not reveal significant differences in kinetic behavior among several of the ADH forms then isolated (23), the present data on $ADH_{Indianapolis}$ and previous studies on π-ADH (2, 20) and ADH_{Bern} (8, 9) clearly demonstrate that different molecular forms of human liver ADH manifest strikingly different catalytic properties. Furthermore, as reported here, the occurrence of enzyme forms with different catalytic properties can vary from individual to individual and between different racial groups. This degree of complexity and genetic heterogeneity can only be unraveled by detailed investigations on each of the individual molecular forms of the human enzyme to provide a rational basis for the elucidation of the biochemistry of human alcohol metabolism and its pathologic derangements.

We are grateful to Suzanne Webb and Leslie Magnes for excellent technical assistance and to Drs. J. Aquilar and J. Benz, Wishard Memorial Hospital, Indianapolis, IN and Dr. J. Lumeng, St. Francis Hospital, Honolulu, HA for collection of liver autopsy specimens. This work was supported by U.S. Public Health Service Grants AA 02342 and GM 15003.

1. Li, T.-K. (1977) *Adv. Enzymol.* **45**, 427–483.
2. Li, T.-K., Bosron, W. F., Dafeldecker, W. P., Lange, L. G. & Vallee, B. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4378–4381.

3. Lange, L. G. & Vallee, B. L. (1976) *Biochemistry* **15,** 4681–4686.
4. Bosron, W. F., Li, T.-K., Lange, L. G., Dafeldecker, W. P. & Vallee, B. L. (1977) *Biochem. Biophys. Res. Commun.* **74,** 85–91.
5. Harada, S., Agarwal, D. P. & Goedde, H. W. (1978) *Hum. Genet.* **40,** 215–220.
6. Bosron, W. F., Li, T.-K. & Vallee, B. L. (1979) *Biochem. Biophys. Res. Commun.* **91,** 1549–1555.
7. Smith, M., Hopkinson, D. A. & Harris, H. (1971) *Ann. Hum. Genet.* **34,** 251–271.
8. von Wartburg, J.-P., Papenberg, J. & Aebi, H. (1965) *Can. J. Biochem.* **43,** 889–898.
9. von Wartburg, J.-P. & Schurch, P. M. (1968) *Ann. N.Y. Acad. Sci.* **151,** 936–946.
10. Stamatoyannopoulos, G., Chen, S.-H. & Fukui, M. (1975) *Am. J. Hum. Genet.* **27,** 789–796.
11. Harada, S., Misawa, S., Agarwal, D. P. & Goedde, H. W. (1980) *Am. J. Hum. Genet.* **32,** 8–15.
12. Teng, Y.-S., Jehan, S. & Lie-Injo, L. E. (1979) *Hum. Genet.* **53,** 87–90.
13. Layne, E. (1957) *Methods Enzymol.* **3,** 447–454.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
15. Hawkins, R. D. & Kalant, H. (1972) *Pharmacol. Rev.* **24,** 67–157.
16. Smith, M., Hopkinson, D. A. & Harris, H. (1973) *Ann. Hum. Genet.* **37,** 49–67.
17. Schenker, T. M., Teeple, L. J. & von Wartburg, J.-P. (1971) *Eur. J. Biochem.* **24,** 271–279.
18. Berger, D., Berger, M. & von Wartburg, J.-P. (1974) *Eur. J. Biochem.* **50,** 215–225.
19. Dubied, A., von Wartburg, J.-P., Bohlken, D. P. & Plapp, B. V. (1977) *J. Biol. Chem.* **252,** 1464–1470.
20. Bosron, W. F., Li, T.-K., Dafeldecker, W. P. & Vallee, B. L. (1979) *Biochemistry* **18,** 1101–1105.
21. Azevedo, E. S., daSilva, M.C.B.O. & Tavares-Neto, J. (1975) *Ann. Hum. Genet.* **39,** 321–327.
22. Smith M., Hopkinson, D. A. & Harris, H. (1973) *Ann. Hum. Genet.* **36,** 401–414.
23. Pietruszko, R., Theorell, H. & deZalenski, C. (1972) *Arch. Biochem. Biophys.* **153,** 279–293.