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# At least six forms of extremely homologous cytochromes P-450 in rat liver are encoded at two closely linked genetic loci

(two-dimensional electrophoresis/peptide maps/allelomorphs/$F_1$ progeny/inducible multigene family)

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**ABSTRACT** A subpopulation of phenobarbital-induced cytochrome P-450 in rat liver has been shown to consist of four closely related forms of the enzyme that appeared to be strain-related. In the present study, polypeptides composing this family were analyzed by two-dimensional electrophoresis of hepatic microsomes from 64 individual phenobarbital-treated rats. The animals surveyed included both sexes from four inbred and five outbred strains/colonies and $F_1$ progenies from 10 crosses. Two new members of this polypeptide family were identified on the basis of their unique electrophoretic behavior and peptide maps. Eight phenotypes were observed that consisted of two to four member polypeptides. The six closely related cytochromes P-450 were found to be encoded at two genetic loci with at least four alleles at the *P-450b* locus and at least two alleles at the *P-450e* locus. Most colonies of outbred strains were characterized by polymorphism at one or both of these loci, and in no case did they contain unique alleles. Analyses of parents and their $F_1$ progenies indicated that the *P-450b* and *P-450e* loci are closely linked on the same autosome and are expressed codominantly. Furthermore, the products of these loci appear to be coordinately regulated. The extreme homology between *P-450b* and *P-450e* genes, their high degree of polymorphism, and their close linkage suggest that they are subject to the same genetic mechanisms that maintain these features in other multigene families.

The versatility of the hepatic monooxygenase system in metabolizing a large number of diverse xenobiotics and endogenous compounds is accounted for by the participation of multiple forms of cytochrome P-450, each of which exhibits different but not absolute substrate specificities. In addition, the population of these hemoproteins is subject to dramatic quantitative and qualitative changes after treatment of animals with a variety of xenobiotics that activate characteristic induction programs (1–4).

At this time, at least eight hepatic cytochromes P-450 have been isolated from either induced or untreated rats (5–11). In several cases, obvious structural differences between various forms of the enzyme were evidenced by peptide mapping, $NH_2$-terminal sequence determinations, and immunochemical methods, which suggested that they are the products of unique and considerably diverse structural genes (7, 8). On the other hand, two-dimensional gel electrophoresis of rat hepatic microsomes revealed a subpopulation of phenobarbital-induced cytochromes P-450 that was microheterogeneous because it consisted of four electrophoretically distinct polypeptides that were characterized by extremely homologous peptide maps and apparent immunochemical identity (12). Subsequently, three of these polypeptides were shown to be coded in distinct mRNAs (13) and were isolated as holoenzymes designated cytochromes P-450bLE, P-450bH, and P-450e (14, 15). Minor structural variations distinguished each of these purified hemoproteins, and comparisons of their spectral and catalytic properties established the functional relationships: cytochrome P-450bLE = P-450bH ≠ P-450e (14, 15). The uniqueness of structural genes for cytochromes P-450bLE and P-450e was substantiated recently when 75% of their amino acid sequences were reported, which revealed substitutions at 10 positions (16). Studies of these closely related cytochromes P-450 also have involved the cloning and sequence determination of three cDNAs. Two of these cDNAs [i.e., pcP-450pb1 and pcP-450pb4, which code for a cytochrome P-450bLE-like protein (16)] evidenced a 100% overlap homology and included ≈93% of the total coding sequence for one mRNA, whereas the remaining cDNA [i.e., pcP-450pb2, which codes for a cytochrome P-450e-like protein (16)] contained ≈63% of the coding sequence for a second mRNA that differed from the first at only 14 of the 921 positions compared (17). A recent extension of this work used these cDNAs as probes to investigate rat DNA, and it was concluded that six gene-like sequences for these closely related cytochromes P-450 exist per haploid genome (18).

A survey for additional members of this closely related family of phenobarbital-induced cytochromes P-450 in rat liver was undertaken in the present study, and two new forms were discovered. In addition, the genetic relationships between these six closely related proteins were established.

## EXPERIMENTAL PROCEDURES

**Source, Treatment, and Breeding of Rats.** Sexually mature male and female rats were obtained from the following sources.

*Outbred strains.* HSD:Sprague–Dawley(SD)BR (Sprague–Dawley rats designated SD-Har) were from Harlan Sprague–Dawley (Indianapolis, IN); Crl:CDH(SD)BR (Holtzman rats designated H-Crl) and Crl:(LE)BR (Long–Evans rats designated LE-Crl) were from Charles River Breeding Laboratories; Blu:(LE)BR (Long–Evans rats designated LE-Blu) were from Blue Spruce Farms (Altamont, NY); Holtzman rats (designated H-Ken) were from a colony at Kent State University that was stocked with rats from Holtzman (Madison, WI).

*Inbred strains.* LEW/CrlBR (Lewis rats designated LEW-Crl), WKY/NCrlBR (Wistar Kyoto rats designated WKY-Crl), BN/CrlBR (Brown Norway rats designated BN-Crl), and CDF(F-344)/CrlBR (Fischer 344 rats designated F344-Crl) were from Charles River Breeding Laboratories. All rats were kept in wire-bottom stainless steel cages, maintained on Purina rodent chow and Milli-Q (Millipore) deionized water provided ad

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Abbreviation: IF, isoelectric focusing.

lib and were subjected to a 12-hr light/dark cycle in a well-ventilated room. Matings of 80-day-old rats were performed in translucent plastic cages containing sawdust, food, and water. The two rats were kept together for not more than 18 days, after which the male was transferred and the female was allowed to whelp in the plastic cage. The pups were weaned at 28 days of age and were maintained for 25 days prior to phenobarbital treatment. Phenobarbital (0.1% in the drinking water) was provided for 4 days, and the rats were starved for the final 24 hr prior to killing by decapitation. The ages of the animals at the time of death for dams, sires, and their progenies were 190 days, 150 days, and 60 days, respectively. The preparation of smooth hepatic microsomes from these rats was as described (19).

**Analytical Methods.** Two-dimensional electrophoresis of microsomes using isoelectric focusing (IF) in the first dimension and $NaDodSO_4$/electrophoresis in the second dimension was performed as described (19). Protein concentrations were assayed by the method of Lowry *et al.* (20) with bovine serum albumin as the standard, and the spectrophotometric procedure of Omura and Sato (21) was used to determine the total content of cytochrome P-450 in microsomes. Polypeptides excised from Coomassie blue-stained IF/$NaDodSO_4$ electrophoretograms were subjected to $^{125}I$-labeled tryptic peptide mapping as described (22).

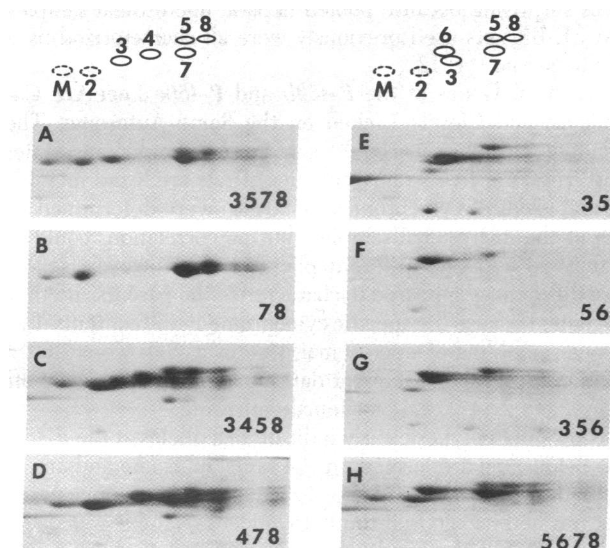


FIG. 1. Portions of IF/$NaDodSO_4$ electrophoretograms for hepatic microsomes from phenobarbital-treated rats, illustrating different phenotypes for closely related forms of cytochrome P-450. The gel portions shown represent a pH range from ≈6.8 to ≈7.5 (right to left), respectively, and an apparent molecular weight region in second-dimensional $NaDodSO_4$ electrophoresis from ≈63,000 to ≈40,000 (top to bottom), respectively. Smooth hepatic microsomes were isolated from animals after phenobarbital treatment, and 100–120 μg of protein was analyzed. (*A*) $F_1$ male from cross 5 (see Table 2). (*B*) $F_1$ male from cross 5. (*C*) $F_1$ male from cross 6. (*D*) $F_1$ male from cross 6. (*E*) $F_1$ female from cross 4. (*F*) Female WKY-Crl. (*G*) $F_1$ female from cross 10. (*H*) $F_1$ male from cross 4. The diagrams indicate the relative positions of identified cytochrome P-450 polypeptides in the gel portions underneath. Polypeptide M (previously designated M2) has been tentatively identified as a male-specific cytochrome P-450 (23). Polypeptide 2 [previously designated PB2 (12)] has been identified as cytochrome P-450PB-1 (9) (unpublished data). Polypeptides 3–8 are closely related forms of phenobarbital-induced cytochromes P-450 [3, 4, and 5 refer to polypeptides PB3–PB5 (12); 6 refers to polypeptide PB6—i.e., cytochrome P-450e (12)]; polypeptides 7 and 8 are new forms discovered in the present study (see Table 1). The relative coordinates for each of the designated polypeptides were verified by coelectrophoresing pairs of microsomal samples. See text for further details.

## RESULTS AND DISCUSSION

**Closely Related Rat Hepatic Cytochromes P-450 Induced by Phenobarbital Consist of at Least Six Forms Encoded at Two Genetic Loci.** Previous studies of phenobarbital-induced, hepatic cytochromes P-450 in rats demonstrated the existence of four closely related but genetically distinct forms of the enzyme that were resolved as microsomal polypeptides (designated PB3–PB6) in two-dimensional electrophoretograms (12, 13). Polypeptides PB3, PB5, and PB6 represent cytochromes P-450bH (15), P-450e (14), and P-450bLE (7), respectively, whereas polypeptide PB4 has not yet been isolated as a holoenzyme. However, these initial studies (12) involved rats representing only a few strains/colonies and used pooled hepatic microsomes from several animals for analyzing polypeptides, which precluded the determination of discrete phenotypes for these closely related hemoproteins.

In the present study, cytochrome P-450 polypeptides in hepatic microsomes from individual phenobarbital-treated rats were analyzed by two-dimensional IF/$NaDodSO_4$ electrophoresis. These animals included males and females from nine strains/colonies and $F_1$ progenies from 10 crosses. Eight distinct phenotypes for these closely related forms of phenobarbital-induced cytochrome P-450 were found, and these are shown in Fig. 1. Previously characterized polypeptides PB3–PB6 were distributed among most of these phenotypes; however, polypeptides PB7 and PB8 were not observed before. In order to certify whether these latter polypeptides represented related forms of the enzyme, they were subjected to *in situ* peptide mapping, which gave the results shown in Fig. 2. The peptide map for polypeptide PB7 was completely homologous with those for polypeptides PB3 (i.e., cytochrome P-450bH) and PB4 (12), whereas the map for polypeptide PB8 was the same as that for polypeptide PB5 (i.e., cytochrome P-450e), which was uniquely characterized by peptide q (12). Evidence that polypeptides PB7 and PB8 represent the products of unique structural genes was recently obtained by using an *in vitro* translation method (13) with mRNA from rats characterized by a 3578 phenotype (unpublished data).

The unique migrations of polypeptides PB3–PB8 indicated in Fig. 1 were verified in several experiments where micro-

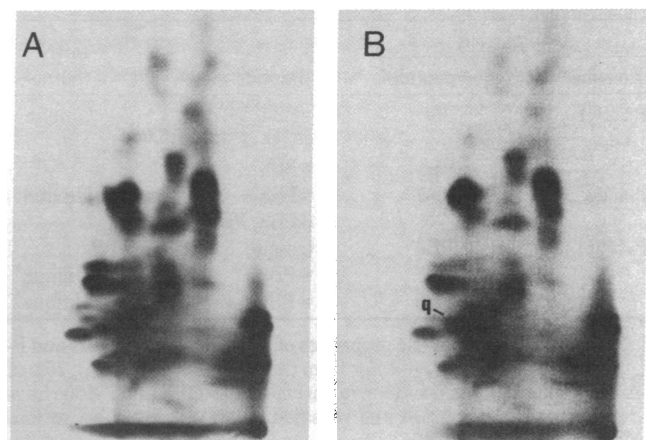


FIG. 2. Radioiodinated tryptic peptide maps for polypeptides PB7 (*A*) and PB8 (*B*). Polypeptides were excised from Coomassie blue-stained IF/$NaDodSO_4$ gels for hepatic smooth microsomes from phenobarbital-treated rats (see Fig. 1). Origins for the maps are in the lower right corners. Electrophoresis was from right (anode) to left (cathode), and chromatography was from bottom to top. The designation "q" in *B* is for a peptide that distinguishes polypeptides PB7 and PB8 (12). Other conditions are as described.

somes from phenotypically distinct rats were mixed prior to IF/$NaDodSO_4$ electrophoresis (data not shown). Because polypeptides PB3 and PB6 migrate closely in these electrophoretograms but have clearly distinct peptide maps (12), the latter procedure was used to verify the 356 and 5678 phenotypes shown in Fig. 1 (data not shown). The characteristics of these six closely related polypeptides and some of their holoenzyme counterparts, summarized in Table 1, provide compelling evidence that polypeptides PB4, PB7, and PB8 are also the products of unique alleles.

To determine the number of genetic loci that express these six closely related forms of cytochrome P-450, several inbred strains of rats were analyzed after phenobarbital-treatment, and it was observed that both male and female BN-Crl, F344-Crl, and LEW-Crl rats were characterized by the 35 phenotype, whereas WKY-Crl rats expressed a 56 phenotype (see examples in Fig. 1). These results demonstrate that polypeptides PB3, PB5, and PB6 are encoded in genes at two chromosomal positions, designated the *P-450b* locus (i.e., alleles for cytochromes P-450bH and P-450bLE) and the *P-450e* locus (i.e., alleles for cytochrome P-450e). The proposed genetic relationships for cytochromes P-450bH, P-450e, and P-450bLE are consistent with their functional characteristics [i.e., on the basis of catalytic and spectral criteria, allelic cytochromes P-450bH and P-450bLE are indistinguishable from each other but differ from nonallelic cytochrome P-450e (14, 15)].

The inheritance patterns for polypeptides PB4, PB7, and PB8 (see below) and their appearance in four-member phenotypes indicates that they also are encoded at either the *P-450b* or *P-450e* loci, with at least one of these forms representing each locus. Thus, the observed 78 phenotype (Fig. 1) requires that polypeptides PB7 and PB8 be encoded at different loci, and the 3458 phenotype requires that genes for polypeptides PB4 and PB7 be allelomorphic.

The peptide map identities summarized in Table 1 strongly indicate that polypeptides PB4 and PB7 are products of the *P-450b* locus and that polypeptide PB8 is a product of the *P-450e* locus. Additional support for these assignments are the relative levels of these polypeptides in microsomes (i.e., P-450e polypeptides exist at lower levels in microsomes than do P-450b polypeptides) and their mobility in the second dimension of IF/$NaDodSO_4$ electrophoresis (i.e., P-450e polypeptides have a lower mobility than P-450b polypeptides) (see Fig. 1). Therefore, it is tentatively concluded that polypeptides PB3, PB4, PB6, and PB7 are products of the *P-450b* locus and that polypeptides PB5 and PB8 are products of the *P-450e* locus.*

Table 1. Molecular characteristics for closely related phenobarbital-induced cytochromes P-450 in rat liver

| Designation | | | |
|---|---|---|---|
| Enzyme* | Polypeptide† | Peptide maps‡ | mRNA coding§ |
| P-450bH | PB3 | =PB4, PB7<br>≠PB5, PB6, PB8 | Unique |
| — | PB4 | =PB3 | — |
| P-450e | PB5 | =PB8<br>≠PB3, PB6 | Unique |
| P-450bLE | PB6 | Unique | |
| — | PB7 | =PB3 | Unique¶ |
| — | PB8 | =PB5 | Unique¶ |

* The spectral and catalytic properties of cytochromes P-450bH and P-450bLE are identical but differ from those for P-450e (14, 15); 75% of the amino acid sequences for cytochromes P-450bLE and P-450e have been directly determined and revealed 10 amino acid substitutions (16).

† Polypeptides PB3–PB8 are characterized by unique coordinates in IF/$NaDodSO_4$ electrophoretograms (see Fig. 1): PB3, PB6, and PB5 have been identified as cytochromes P-450bH, P-450bLE, and P-450e, respectively (14, 15).

‡ Radioiodinated tryptic peptide maps for polypeptides PB3–PB8 were determined after their resolution in IF/$NaDodSO_4$ electrophoretograms, and they *all* exhibited ≥95% homology (ref. 12; see Fig. 2).

§ See ref. 13.

¶ Unpublished data.

Considering the small number of *Rattus norvegicus* tested to date, it is possible that additional allelomorphic forms of the enzyme exist in this species. Even without additional forms, 30 phenotypes are possible when all combinations of four different alleles at the *P-450b* locus and two alleles at the *P-450e* locus are considered. Our previous observation of only polypeptides PB3, PB4, and PB5 in *pooled* hepatic microsomes from phenobarbital-treated H-Ken rats (12, 19) suggests the existence of 345 and 45 phenotypes in addition to those reported here. Therefore, approximately one-third of the expected phenotypes already have been detected. The present results indicate that none of the five outbred colonies of rats tested are exclusively characterized by particular alleles, and four of these colonies contained individuals that were heterozygous at the *P-450b* or *P-450e* locus or at both loci. In this regard, all of the H-Crl rats analyzed to date were characterized by a 35 phenotype. However, the demonstration that some individual LE-Blu rats possess a 356 phenotype (unpublished experiments; see Table 2) was surprising because pooled hepatic microsomal samples from LE-Blu rats used previously were all characterized by a 56 "phenotype" (4, 12).

**Structural Genes at the *P-450b* and *P-450e* Loci Are Codominant and Closely Linked on the Same Autosome.** The phenotypes for phenobarbital-treated parents and $F_1$ progenies of 10 crosses of five strains/colonies of rats are listed in Table 2. Total levels of cytochromes P-450 also were determined for each of the microsomal samples, but no correlations could be established with the different phenotpyes. However, sex-related differences appeared to characterize the parents and their progenies because the specific cytochrome P-450 contents (i.e., average nmol/mg of microsomal protein ± SD) were 2.69 ± 0.34 for sires, 2.28 ± 0.36 for dams, 3.10 ± 0.33 for male offspring, and 2.14 ± 0.41 for female offspring.

The $F_1$ phenotypes clearly indicate that alleles at the *P-450b* and *P-450e* loci are located on the same autosome and are expressed codominantly. Crosses 1–6 involved at least one parent that was heterozygous at the *P-450b* locus or the *P-450e* locus, or at both, but the resulting 36 $F_1$ offspring of these crosses consisted of only parental combinations and no recombinants. For the small sample tested, the parental haplotypes (i.e., haploid genotypes), defined in terms of the polypeptide products encoded at the *P-450b–P-450e* loci, would be: *PB3–PB5*, *PB6–PB5*, *PB7–PB8*, and *PB4–PB8*. Considering a binomial distribution for the 36 parental and zero recombinant $F_1$ phenotypes detected, there is a 90% probability that the distance between the *P-450b* and *P-450e* loci is less than or equal to 6.2 map units and a 30% probability that this distance is less than or equal to 1.0 map unit.

**The Synthesis of P-450b and P-450e Polypeptides Is Coordinately Regulated.** Under a variety of conditions, P-450b and P-450e polypeptides were always coinduced, and the level

* If the assignments of alleles for polypeptides PB4, PB7, and PB8 were reversed (i.e., contrary to compelling circumstantial evidence), then polypeptides PB3, PB6, and PB8 would be encoded at the *P-450b* locus and polypeptides PB4, PB5, and PB7 would be encoded at the *P-450e* locus. Phenotypes that might have resolved this minor uncertainty (e.g., 45, 38, 457, and 368, etc.) were not observed among the limited number of rats used in the present study.

Table 2. Parental and $F_1$ progeny phenotypes for closely related phenobarbital-induced cytochromes P-450 in rat liver

| Parents* | | | Progeny† | | | | |
|---|---|---|---|---|---|---|---|
| Strain (phenotype) | | | Observed | | | Theoretical‡ | |
| Male | ×§ | Female | No. | Sex | Phenotype | Phenotype | Ratio |
| SD-Har (3578) | 1 | H-Crl (35) | 2 | M | 35 | 35 | 1 |
| | | | 2 | M | 3578 | 3578 | 1 |
| | | | 2 | F | 3578 | | |
| H-Crl (35) | 2 | SD-Har (3578) | 2 | F | 35 | 35 | 1 |
| | | | 1 | M | 3578 | 3578 | 1 |
| SD-Har (3578) | 3 | LE-Crl (356) | 1 | M | 35 | 35 | 1 |
| | | | 1 | F | 35 | 3578 | 1 |
| | | | 1 | M | 3578 | 5678 | 1 |
| | | | 1 | M | 356 | 356 | 1 |
| LE-Blu (356) | 4 | SD-Har (3578) | 3 | F | 35 | 35 | 1 |
| | | | 1 | F | 3578 | 3578 | 1 |
| | | | 1 | M | 5678 | 5678 | 1 |
| | | | 1 | F | 5678 | 356 | 1 |
| H-Ken (3578) | 5 | SD-Har (78) | 1 | M | 78 | 78 | 1 |
| | | | 1 | F | 78 | 3578 | 1 |
| | | | 1 | M | 3578 | | |
| LE-Crl (3458) | 6 | SD-Har (3578) | 2 | M | 35 | 35 | 1 |
| | | | 1 | F | 35 | 3578 | 1 |
| | | | 2 | M | 3578 | 3458 | 1 |
| | | | 3 | F | 3578 | 478 | 1 |
| | | | 2 | M | 3458 | | |
| | | | 4 | M | 478 | | |
| SD-Har (35) | 7 | H-Ken (35) | 1 | F | 35 | 35 | |
| SD-Har (35) | 8 | LE-Blu (56) | 2 | M | 356 | 356 | |
| | | | 4 | F | 356 | | |
| LE-Blu (56) | 9 | H-Crl (35) | 3 | M | 356 | 356 | |
| | | | 3 | F | 356 | | |
| H-Crl (35) | 10 | LE-Blu (56) | 2 | M | 356 | 356 | |
| | | | 5 | F | 356 | | |

Designations for source and strain of rats are found under *Experimental Procedures*. Different individuals were mated for each cross. Numerical designations for the phenotypes refer to polypeptides PB3–PB8, which were analyzed by IF/$NaDodSO_4$ electrophoresis of hepatic microsomes from phenobarbital-treated animals as shown in Fig. 1.

* Dams were analyzed at 190 days of age, which was at least 25 days after their offspring were weaned; sires were analyzed at 150 days of age.

† Ten pups were apparently subject to maternal cannibalization: the total surviving progeny consisted of 38 males and 34 females. These were analyzed at 60 days of age with the exception of 16 offspring from the last three crosses.

‡ The theoretical ratios of phenotypes assumed that the *P-450b* locus (coding allozymic polypeptides PB3, PB4, PB6, and PB7) is closely linked with the *P-450e* locus (coding allozymic polypeptides PB5 and PB8) such that genetic crossing-over between these loci is a rare event that results in the persistence of parental combinations in the $F_1$ progeny (see text for details).

§ Designation for the crosses.

of the former in hepatic microsomes was consistently ≈2 times greater than that of the latter. Because these characteristics were observed for outbred rats (homozygous at the *P-450b* and *P-450e* loci) after their treatment with five different inducing agents (4) and were independent of age and sex for phenobarbital-treated inbred rats (23), it was concluded that *P-450b* and *P-450e* gene products are coordinately regulated (4). Examination of the relative levels of polypeptides PB3–PB8, which characterize the eight phenotypes shown in Fig. 1 (i.e., PB3 ≃ PB4 ≃ PB6 ≃ PB7 > PB5 ≃ PB8), indicate that these characteristics can be extended to include all allelomorphic forms of the enzyme. In view of the recent evidence that P-450b and P-450e polypeptides have similar turnover rates (24) and that their corresponding mRNA levels had been shown to be substantially increased in liver after treatment with inducing agents (13, 25–28), it is likely that the coordinate regulation of these cytochromes P-450 occurs at the level of transcription or pre-mRNA processing. In this regard, it is possible that the proximity of the *P-450b* and *P-450e* loci on the same chromosome (see above) may be important for their coregulation.

**Implications and Biological Significance.** Because the *P-450b* locus and the *P-450e* locus are now known to be polymorphic, it is possible that previous studies might have been complicated by heterozygosity at these loci. For example, two recent investigations used pooled hepatic microsomes from outbred Sprague–Dawley rats and attempted to quantify cytochromes $P\text{-}450_{PB\text{-}B}$ [correlated with cytochrome P-450b (8)] and $P\text{-}450_{PB\text{-}D}$ [correlated with cytochrome P-450e (8)] by using one-dimensional IF of microsomes to resolve polypeptides for immunoquantitation (3, 29). Because nonallelic polypeptides PB5 and PB7 are found in SD-Har rats (Table 2) and are not resolved by IF (Fig. 1), it now appears that these reported levels of cytochromes $P\text{-}450_{PB\text{-}B}$ and $P\text{-}450_{PB\text{-}D}$ are probably not accurate. In fact, this conclusion is supported by the data in these studies because significantly different results were found for the absolute and relative levels of these hemoproteins in hepatic microsomes from phenobarbital-treated SD-Har rats (3) when compared with similarly treated SD rats from another colony (29).

The proposed amino acid sequences for cytochromes P-450bLE and P-450e isolated from LE-blu rats (i.e., polypeptides PB6 and PB5, respectively) (16) represent an informational composite, 75% of which was determined from direct amino acid sequence determination, 25% having been interpolated by using cDNA information from Sprague–Dawley rats (17). If these proposed sequences are correct, then (*i*) polypeptide PB6 should be present in hepatic microsomes from the phenobarbital-treated Sprague–Dawley rats used to prepare the cDNAs (17), and (*ii*) no charge differences exist between polypeptides PB6 and PB5, even though they are completely resolved as such after IF (see Fig. 1*F*) or as holoenzymes after DEAE chromatography (14). In view of the present study, these apparent discrepancies might be rationalized if the cDNA information used to complete the amino acid sequences of cytochromes P-450bLE and P-450e was for polypeptides PB7 and PB5, respectively, because these variant forms are found in Sprague–Dawley rats (Table 2) and show no charge differences after IF (Fig. 1). Therefore, it might be concluded that the composite amino acid sequence proposed for cytochrome P-450e is correct, but that proposed for cytochrome P-450bLE is incorrect at one or more interpolated residues between positions 159 and 232 and between positions 275 and 323 (16). In this regard, the number of amino acid substitutions among the four allelomorphic products of the *P-450b* locus is unknown, but it seems that at least two amino acid charge differences are involved because polypeptides PB3, PB4, and PB7 were resolved by IF after a mixture of microsomes from rats with 478 and 35 phenotypes was submitted to IF/$NaDodSO_4$ electrophoresis (data not shown, but see Fig. 1).

Three previous examples of allelic variants of cytochromes P-450 were established primarily on the basis of their different catalytic properties (30–32), but cytochromes P-450bLE and P-450bH appear to be indistinguishable in this regard (15). This latter finding might indicate that polymorphism at the *P-450b* and *P-450e* loci was not determined by selection and is simply the result of genetic drift. However, it is quite possible that appropriate substrates, which might discriminate between these allelomorphic forms of the enzyme, have not yet been tested. It is also possible that unknown aspects of their functionality might have provided a basis for selection of these variant membrane-bound hemoproteins.

In view of finding six genes or gene-like sequences for this closely related family of cytochromes P-450 per haploid genome in rats, the possibility of pseudogene members was suggested (18). Because the present study indicates the existence of only *two* loci that express these related forms of the enzyme in the liver, it appears that this suggestion may be true. However, it is still possible that more than two authentic genes are present whose polypeptide products escaped detection in our electrophoretic analysis, either by existing at low levels in microsomes or by sharing the same electrophoretic and peptide map characteristics as one of polypeptides PB3–PB8. It is also possible that other loci are expressed in nonhepatic tissues.

*P-450b* and *P-450e* genes have several characteristics in common with those for H-2(D,K,L) antigens, which are part of the major histocompatibility complex in the mouse (33–35), because both of these multigene families (*i*) are clustered and codominantly expressed on one autosome, (*ii*) contain extremely homologous genes that are characterized by extensive polymorphism, and (*iii*) possibly coexist with pseudogenes.[†] This parallelism does not necessarily mean that the multiplicity of structural genes for rat cytochromes P-450 and H-2 antigens in the mouse are comparable (36) but suggests that the same genetic mechanisms that maintain sequence homologies among closely linked genes and their extensive polymorphism may be operative in both cases. The importance of such mechanisms regarding the divergent and cohesive evolution of multigene families has been discussed recently (35, 37).

[†] It is probably coincidental, but the products of these two multigene families are integral membrane polypeptides characterized by similar sizes (i.e., $M_r$ 50,000 ± 10,000).

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