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# Identification of the Form of Cytochrome *P*-450 Induced in Neonatal Rabbit Liver Microsomes by Phenobarbital

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SUMMARY

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Transplacental induction of cytochrome P-450 by phenobarbital was studied in neonatal rabbit liver microsomes. This treatment increased a single form of cytochrome *P*-450 identified by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, peptide mapping and immunological characterization as cytochrome *P*-450, form 2. Form 2 is the predominant species of the cytochrome in adult liver microsomes from rabbits pretreated with phenobarbital. This assignment was further supported by examining the kinetics of 7-ethoxycoumarin O-deethylation. Reconstituted form 2 and phenobarbital-induced neonatal microsomes showed a similar monophasic dependence on 7-ethoxycoumarin concentration. Phenobarbital-induced adult microsomes, however, exhibited a biphasic dependence on substrate concentration. This is apparently due to the presence of other forms of cytochrome *P*-450 in these microsomes participating in the O-deethylation of this substrate. Previously an age difference was found to exist in the induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of two forms of cytochrome *P*-450, forms 4 and 6, in rabbit liver microsomes. Form 6 is the sole form induced in the neonate, whereas form 4 is the major form and form 6 is the minor form induced in the adult rabbit liver. Thus, phenobarbital, like 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, induces only one form of cytochrome *P*-450 in neonatal liver microsomes. Unlike 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, phenobarbital induces the same major form in both adults and neonates.

## INTRODUCTION

The occurrence of multiple forms of cytochrome *P*-450 in the endoplasmic reticulum is dependent on factors that include species, tissue type, age and exposure to inducing agents (1). Previously, this laboratory reported on the age-dependent induction of cytochrome *P*-450 by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[1] in rabbit liver (2). In the adult rabbit, two forms, 4[2] and 6, are induced by TCDD, with form 4 having the greater concentration of the two. In the neonate rabbit, transplacental induction by TCDD increases a single species of cytochrome *P*-450 against a background of low levels of cytochrome *P*-450 normally encountered in newborns. This component was identified as form 6 by both physical and functional criteria (2). These differences in the response of adult and neonate rabbits to TCDD suggest that an age-dependent factor(s) controls the induction of these two forms.

In the present study, we examined what age-related factors may be involved in PB induction. In adult rabbit liver, PB treatment resulted in the induction of a major form of cytochrome *P*-450, form 2 (3). Previous investigations concerned with the treatment of neonatal rabbits with PB during fetal life have resulted in the observations that cytochrome *P*-450 levels are elevated and that the metabolic capacities of these liver microsomes are altered relative to untreated neonate microsomes (4–6).

By employing SDS–PAGE, peptide mapping experiments, immunological criteria and catalytic studies, we have identified the major microsomal cytochrome *P*-450 component, responsible for the elevated cytochrome *P*-450 content and altered metabolism, as form 2.

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[1] The abbreviations used are: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; PB, phenobarbital; SDS–PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

[2] In compliance with recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature suggesting a numerical system based on gel electrophoresis be used to designate multiple enzyme forms, we have employed the following system of nomenclature for multiple forms of rabbit cytochrome *P*-450. The major phenobarbital-induced species ($M_r$ = 48,500) is designated form 2, the constitutive species ($M_r$ = 51,000) is designated form 3, the major TCDD-induced species ($M_r$ = 54,500) is designated form 4, and the other TCDD-induced species ($M_r$ = 57,000) is designated form 6.



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Thus, PB, like TCDD, induces to a predominant degree a single form of cytochrome *P*-450 in neonate hepatic microsomes. Unlike the finding with TCDD, the major form induced by PB in neonate liver is also the major form induced in adult liver microsomes.

## MATERIALS

Phenobarbital, NADPH (tetrasodium salt), cytochrome *c* (horse heart type III) and crystalline bovine serum albumin (Fraction V) were obtained from Sigma. 7-Ethoxycoumarin (gold label), benzo[*a*]pyrene (gold label), biphenyl and 4-hydroxybiphenyl were purchased from Aldrich (the latter two compounds were further purified by recrystallization from petroleum ether). 7-Ethoxyresorufin and resorufin were from Pierce Chemical Company. Umbelliferone (7-hydroxycoumarin, practical grade) was from J. T. Baker Chemical Company and was further purified by sublimation. Benzphetamine was a gift from Upjohn Company. All materials for SDS–PAGE were obtained from Bio-Rad, except Coomassie brillant blue (Sigma). 3-Hydroxybenz[*a*]pyrene was kindly supplied by Dr. Harry Gelboin (National Cancer Institute). TCDD was given to us by the Dow Chemical Company. All other chemicals and chromatography media were of the highest, commercially available grade.

## METHODS

*Animals.* Pregnant New Zealand white rabbits (3–4 kg) were given PB in their drinking water (0.1% as the sodium salt) continuously from the 24th day following conception. Newborn rabbits were killed within 12 hr of birth (birth normally occurred on the 31st or 32nd day of gestation). TCDD- and nontreated (control) neonates were obtained as described (2). TCDD-, PB- and nontreated adult rabbits were handled according to the methods of Johnson *et al.* (7).

*Microsomes.* Adult liver microsomes were prepared by the method of van der Hoeven and Coon (8) and suspended in 50 mM potassium phosphate, pH 7.4, 20% glycerol and 0.1 mM $Na_2EDTA$. Neonate microsomes were isolated as above with the exceptions described (2) and suspended in the same buffer as the adult microsomes.

*Purification of individual forms of cytochrome P-450 and NADPH–cytochrome P-450 reductase.* Forms 4 and 6 were isolated from liver microsomes of TCDD-treated adult rabbits according to the procedure of Johnson and Muller-Eberhard (9, 10). The form 6 preparation was purified further as described by Johnson.[3] Form 2 and NADPH–cytochrome *P*-450 reductase were purified from PB-treated adult rabbits (7). Form 3 was prepared from control adult rabbits by the method of Johnson.[3] The average specific content of cytochrome P-450 among the various preparations used in the studies reported here were: form 4, 17.8 nmole $mg^{-1}$; form 6, 16.1 nmole $mg^{-1}$; form 2, 17.5 nmole $mg^{-1}$; and form 3, 16.3 nmole $mg^{-1}$. The average specific activity of the NADPH–cytochrome *P*-450 reductase was 50 units $mg^{-1}$ (one unit defined as one micromole of cytochrome *c* reduced per minute per milligram of protein).

[3] Johnson, E. F., *J. Biol. Chem.* **255**:304–309 (1980).

*Protein, cytochrome P-450 and reductase determinations.* Protein concentrations were estimated by the method of Lowry *et al.* (11) as modified by Bensadoun and Weinstein (12). Crystalline bovine serum albumin served as the standard. Cytochrome *P*-450 content was determined from the difference spectrum of CO-dithionite-reduced cytochrome *P*-450, using an extinction coefficient of 91 $mM^{-1}$ $cm^{-1}$ (13). NADPH–cytochrome *P*-450 reductase activity was determined as described in an earlier publication from this laboratory (14).

*Polyacrylamide gel electrophoresis and peptide mapping studies.* Polyacrylamide gel electrophoresis was performed in the presence of sodium dodecyl sulfate (15). Densitometric scans of SDS–PAGE electrophoretograms, stained with Coomassie brilliant blue, were recorded at 580 nm on a Beckman spectrophotometer equipped with a Gilford Linear Transport 2410-S.

Peptide maps were obtained by the method of Cleveland *et al.* (16) with modifications for the proteolysis of peptides recovered from gel slices (Method II) (17).

*Immunological techniques.* Antibody to form 3 was prepared as described by Johnson.[3] Antibody to form 2 was prepared in a manner similar to that described for the production of antibodies to forms 4 and 6 (2, 14). Goats were immunized with a homogeneous form 2 antigen (16 μg/injection) obtained from a highly purified preparation, utilizing the electrophoretic elution method of proteins from SDS–PAGE described by Johnson *et al.* (17). An anti-form 2 immunoglobulin fraction was isolated from the immune serum by DEAE-cellulose chromatography and characterized by immunodiffusion (14). Immunological characteristics of the various microsomal preparations were also determined by the immunodiffusion method (18). Control antibody (nonimmune goat serum) was produced by the procedure of Johnson *et al.* (14).

*Enzyme assays.* The procedure for reconstitution of purified forms of cytochrome *P*-450 with NADPH–cytochrome *P*-450 reductase and lipid has been described previously (7). 7-Ethoxycoumarin hydroxylase activity was assayed by the method of Ullrich *et al.* (19) using the extraction procedure for the product 7-hydroxycoumarin (umbelliferone) described by Creaven *et al.* (20). Biphenyl 4-hydroxylation was measured according to the method of Johnson *et al.* (7). Benzo[*a*]pyrene 3-hydroxylase activity was analyzed by the procedure of Nebert and Gelboin (21). 7-Ethoxyresorufin O-deethylation was assayed as described by Burke *et al.* (22). Benzphetamine demethylase activity was estimated by formaldehyde production by the methods employed by Nash *et al.* (23).

## RESULTS

Neonatal rabbits, exposed transplacentally to PB during the last week of gestation, exhibit a threefold increase in cytochrome *P*-450 content relative to nontreated neonates (Table 1). PB treatment of adult rabbits induces a twofold increase in cytochrome *P*-450 content over that observed in control adults. The reductase levels are also increased in adult rabbits treated with PB (Table 1).

A typical analysis on SDS–PAGE of the various microsomal preparations (PB- and nontreated adults and

TABLE 1

*Induction of rabbit liver microsomal cytochrome P-450 and related enzymes by phenobarbital in adult and newborn animals*[a]

Assays were conducted as described under Methods.

| | Source of microsomes | | | |
|---|---|---|---|---|
| | Newborn | | Adult | |
| | Control | PB | Control | PB |
| Cytochrome *P*-450[b] | 0.3 ± 0.1 | 0.9 ± 0.2 | 1.8 ± 0.2 | 3.6 ± 0.5 |
| NADPH–cytochrome *P*-450 reductase[c] | 0.13 ± 0.13 | 0.23 ± 0.07 | 0.23 ± 0.04 | 0.32 ± 0.03 |
| Benzphetamine[d] | nil | 12.1 ± 1.8 | 3.8 ± 0.3 | 4.3 ± 0.2 |
| Biphenyl 4-hydroxylase[d] | 0.9 ± 0.6 | 2.5 ± 0.4 | 1.0 ± 0.2 | 2.4 ± 0.1 |
| Benzo[*a*]pyrene hydroxylase[d] | nil | 0.11 ± 0.01 | 0.08 ± 0.01 | 0.11 ± 0.01 |
| 7-Ethoxyresorufin *O*-deethylase[d] | <0.04 | 0.11 ± 0.02 | 0.13 ± 0.04 | 0.04 ± 0.01 |

[a] Mean ± SD for $N \geq 4$.
[b] nmole/mg protein.
[c] μmole cytochrome *c* reduced/min/mg protein.
[d] moles product formed/mole of cytochrome *P*-450/min.

neonates) and four highly purified forms of cytochrome *P*-450 (forms 2,3,4 and 6) is illustrated in Fig. 1. In both PB-treated neonates and adults, the component migrating with the same mobility as form 2 appears to be induced. This point is more clearly depicted in the densitometric scans of the 48,000 to 65,000 molecular weight region of the SDS–PAGE, as shown in Fig. 2. In neonates, treatment with PB or TCDD results in the induction of a single component against a background of low peptide levels. In the TCDD-neonate,[4] the induced component has previously been identified as form 6 (2). In the PB-neonate the induced fraction has a mobility similar to that of form 2. Scans of PB-, TCDD- and nontreated adults (Fig. 2) demonstrate that these inducers increase specific peptides in the cytochrome *P*-450 molecular weight region. The identification of these components has been described previously (1).

Peptide mapping experiments provide a useful means of differentiating individual forms of cytochrome *P*-450 based on the pattern of peptide fragments produced by partial proteolytic digestion of the protein under investigation. Each of the four forms tested in this manner, forms 2,3,4 and 6, exhibit a unique pattern of peptide fragments (17). By resolving the principal protein component in PB-neonates from the other microsomal proteins by using SDS–PAGE and excising this component from the gel matrix, as described in the Methods section, and reacting the protein with the protease(s), we have a means of comparing the resulting pattern of peptides obtained from microsomes with those of the individual purified forms. As shown in Fig. 3, the patterns of fragments produced from each of three proteases, papain, α-chymotrypsin and *Staphylococcus aureus* $V_8$, are nearly identical between the induced component in PB-neonates and purified form 2.

The presence of form 2 in PB-neonate microsomes was confirmed when they were characterized immunologically with antibody to form 2 (Fig. 4). Initially, antibody directed against form 2 was tested with purified preparations of forms 2,3,4 and 6. Nonimmune immunoglobulin was also included as a control. As illustrated in Fig. 4, antibody to form 2 reacted only with form 2 antigen, indicating monospecificity. Solubilized liver microsomal fractions from TCDD-, PB- and nontreated adults and neonates were then tested with the antibody to form 2. Strong precipitin lines formed between the antibody and both PB-neonate and adult microsomes, indicating the presence of form 2 in these preparations. Nontreated adult microsomes also exhibited a response, although much weaker. Non-treated neonate microsomes and TCDD-neonate and adult microsomes failed to react with the antibody.

Having established the presence of form 2 in PB-neonate microsomes by the aforementioned criteria, we examined the functional properties of the PB- and nontreated neonate and adult microsomal preparations (Table 1). With each substrate tested, treatment of neonates

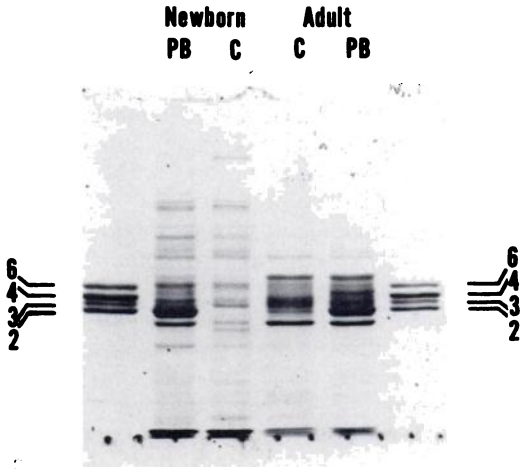


FIG. 1. *SDS–PAGE of newborn and adult rabbit microsomes and purified forms of cytochrome P-450*

SDS–PAGE was performed as described under Methods. Electrophoretic migration was from top to bottom; 30 μg of each microsomal preparation and 2 μg of each purified form of cytochrome *P*-450 were applied to the wells of a 7.5% polyacrylamide analytical gel. The dimensions of each track were 8 × 100 × 3 mm. **C** and **PB** denote control and phenobarbital-induced microsomes, respectively. **2,3,4** and **6** denote the individual purified forms of cytochrome *P*-450, forms 2,3,4 and 6, respectively.

[4] As a matter of convenience, animals pretreated with an inducer will be prefixed with the abbreviation for that inducer. For example, PB-neonates will refer to neonatal rabbits pretreated with phenobarbital.

with PB effects a higher rate of metabolism relative to untreated neonates. Moreover, PB-neonate microsomes catalyze the N-demethylation of benzphetamine, a form 2 marker, with a turnover number greatly exceeding that of PB-adult microsomes.

In reconstitution experiments (Table 2), form 2 was found to O-deethylate 7-ethoxycoumarin at a rate 10 and 20 times greater than forms 6 and 4, respectively. Furthermore, form 2 exhibits an apparent $K_m$ 10 times greater than form 6 or 4. Reconstitution of form 3 results in activities at the lower limits of detection (less than 50 pmole $min^{-1}$ $nmole^{-1}$) thus precluding the determination of a $K_m$.

In Table 3, the kinetic parameters of control, PB- and TCDD-treated adult and neonate microsomes in the metabolism of 7-ethoxycoumarin are listed. In parallel with the reconstitution results, PB treatment shows a greater apparent $V_{max}$ in both adults and neonates as compared with their nontreated counterparts. While the PB-neonate microsomes show a monophasic dependence on substrate concentration (Fig. 5), in agreement with purified form 2, PB-adult microsomes exhibit a biphasic dependence with respect to substrate concentration. The low affinity $K_m$ is in agreement with that obtained for form 2 and PB-neonate microsomes, whereas the high affinity $K_m$ agrees with that observed for forms 4 and 6,

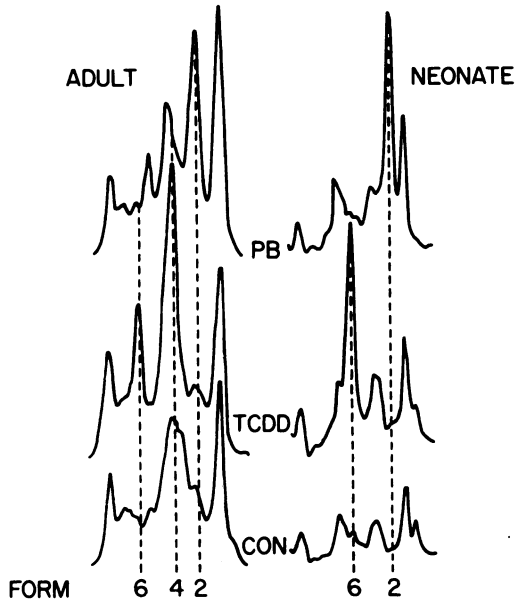


FIG. 2. *Densitometric scans of the 45,000 to 65,000 molecular weight region of rabbit liver microsomes resolved on SDS–PAGE*

The procedure is described under Methods. Electrophoretic migration was from left to right. The amount of proteins used are the same as in Fig. 1. Dashed lines indicate electrophoretic mobilities associated with forms 2,4 and 6.

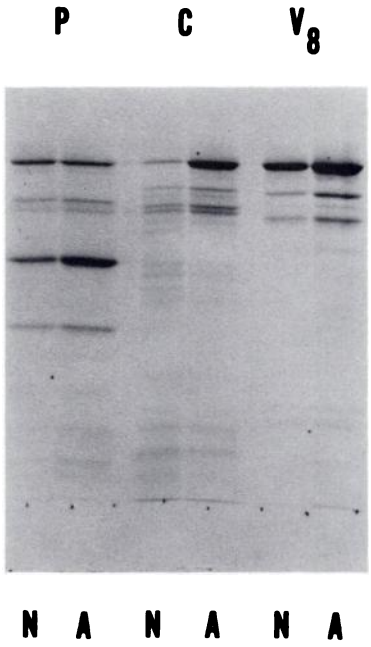


FIG. 3. *Peptide mapping experiments*

The procedure is described under Methods. Electrophoretic migration was from top to bottom. **N** denotes PB-neonate microsomes. **A** denotes purified form 2. The proteases used were: **P**, papain; **C**, α-chymotrypsin; $V_8$, *S. aureus* $V_8$.

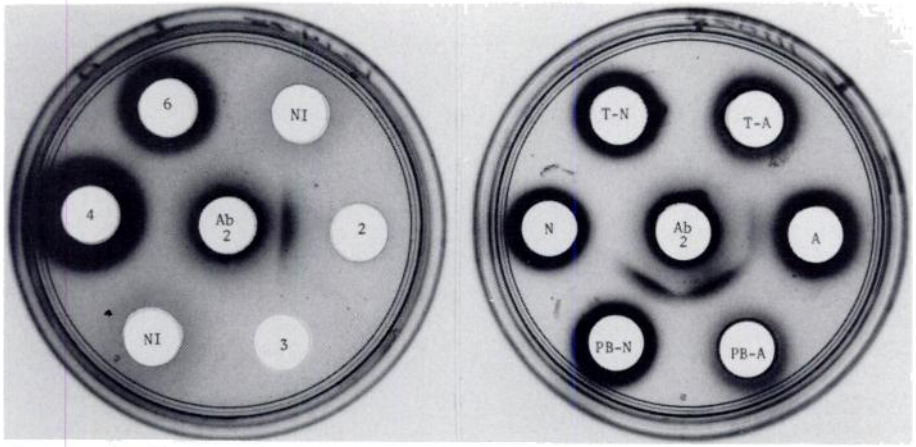


FIG. 4. *Immunodiffusion experiments*

Each well contains 100 μl of the following: **NI**, nonimmune goat plasma, **2**, form 2 (2.4 μM); **3**, form 3 (6.5 μM); **4**, form 4 (16.9 μM); **6**, form 6 (10.6 μM); **N**, microsomes from untreated neonates (1.0 μM); **PB-N**, microsomes from PB-treated neonates (3.3 μM); **T-N**, microsomes from TCDD-treated neonates (7.0 μM); **A**, microsomes from untreated adults (7.7 μM); **T-A**, microsomes from TCDD-treated adults (7.6 μM); **PB-A**, microsomes from PB-treated adults (6.5 μM); and $Ab_2$, antibody directed against form 2.

TABLE 2

*Kinetic properties of 7-ethoxycoumarin O-deethylation catalyzed by three reconstituted forms of cytochrome P-450*[a]

Assays were conducted as described under Methods. $K_m$ and $V_{max}$ were obtained by plotting $1/v$ vs $1/s$.

| Form | $V_{max}$[b] ($min^{-1}$) | $K_m$ ($\mu M$) |
|---|---|---|
| 2 | 1.01 ± 0.06 | 210 ± 20 |
| 4 | 0.05 ± 0.03 | 19 ± 6 |
| 6 | 0.11 ± 0.03 | 21 ± 12 |

[a] Mean ± SD for $N \geq 4$.
[b] Moles umbelliferone formed/min/mole cytochrome *P*-450.

TABLE 3

*The effect of phenobarbital and 2,3,7,8-tetrachlorodibenzo-p-dioxin pretreatment on microsomal 7-ethoxycoumarin O-deethylase*[a]

Assays were performed as described under Methods. $K_m$ and $V_{max}$ were obtained by plotting $1/v$ vs $1/s$.

| Inducer | Age | $V_{max}$[b] ($min^{-1}$) | $K_m$ ($\mu M$) |
|---|---|---|---|
| None | Adult | 0.10 ± 0.03 | 21 ± 10 |
| PB | Adult | 0.33 ± 0.15 | 200 ± 70 |
| | | 0.10 ± 0.02 | 22 ± 5 |
| TCDD | Adult | 0.06 ± 0.02 | 21 ± 10 |
| None | Neonate | nil | — |
| PB | Neonate | 0.67 ± 0.30 | 200 ± 30 |
| TCDD | Neonate | 0.05 ± 0.03 | 21 ± 11 |

[a] Mean ± SD for $N \geq 4$.
[b] moles umbelliferone formed/min/mole cytochrome *P*-450.

control adult and TCDD-pretreated adult and neonate microsomes. Control neonate microsomes exhibit little activity toward 7-ethoxycoumarin.

## DISCUSSION

The results obtained in this investigation illustrate three important points with respect to cytochrome *P*-450 and cytochrome *P*-450-mediated metabolism. First, we have established the identity of the form of cytochrome *P*-450 that predominates in PB-pretreated neonate hepatic microsomes. Second, evidence is provided that the appearance and expression of multiple forms of cytochrome *P*-450 are regulated by an age-dependent process. Lastly, the concept that individual forms of cytochrome *P*-450 are significant determinants of microsomal metabolism, is further substantiated.

Following transplacental exposure to PB, neonate liver microsomes contain elevated levels of cytochrome *P*-450 relative to nontreated neonates. Concomitant with this increase in cytochrome *P*-450 content, there is an enhancement in the metabolism toward certain substrates by the PB-neonate microsomes. The demonstration that these increases are the result of the induction of a specific form (or forms) of cytochrome *P*-450 is contingent on the ability to identify that form(s).

We have isolated and purified four distinct forms of cytochrome *P*-450: forms 2,3,4 and 6 from adult liver microsomes. Several criteria have been developed in this laboratory with which to partially characterize and distinguish each of these four forms on the basis of their inherent physical and functional properties. These include differences in apparent molecular weights, peptide maps, immunological properties and enzymatic activities. Each of these analytical techniques can be applied not only to individual purified forms but also to those forms as they exist in crude mixtures, as is the case in microsomal preparations. Thus, we have a means of comparison between individual forms of isolated cytochrome *P*-450 and microsomal cytochrome *P*-450 in tissue samples.

SDS–PAGE provides an efficient experimental technique whereby a complex mixture of peptides can be resolved and distinguished from one another on the basis of their relative electrophoretic mobilities. These mobilities are generally characteristic of a particular molecular weight. Densitometric scans of SDS–PAGE can in turn indicate the relative concentrations of peptides in a given molecular weight region. SDS–PAGE is therefore useful in providing evidence for the occurrence and induction of multiple forms of cytochrome *P*-450.

The possibility arises, however, that what appears as a single peptide component may actually be two or more peptides with similar mobilities. Furthermore, there is no reliable means by which we can readily ascertain whether a given peptide is indeed a form of cytochrome *P*-450 on the basis of its relative mobility alone. Thus, we observe that following PB pretreatment, neonate microsomes contain a single peptide or group of peptides that are increased in concentration relative to untreated neonates with a mobility equal to that of purified form 2. In addition, this increase occurs with a mobility corresponding to that at which adult microsomes exhibit an increase in the concentration of form 2, following PB treatment. In order to corroborate these observations a form-specific technique is required.

Peptide mapping experiments are useful in this respect in as much as this technique reflects upon the primary structure of a protein. The protein under investigation is subjected to limited proteolysis by a given protease in the presence of SDS. The resulting peptide fragments are then analyzed by SDS–PAGE. Each of our four purified forms of cytochrome *P*-450 produce a characteristic, reproducible pattern of peptide fragments when analyzed in this manner (17). This procedure can also be applied to an analysis of peptides excised from SDS–PAGE, enabling a comparison between the peptide maps of a microsomal protein component with those of the purified forms. The possibility exists, however, that a

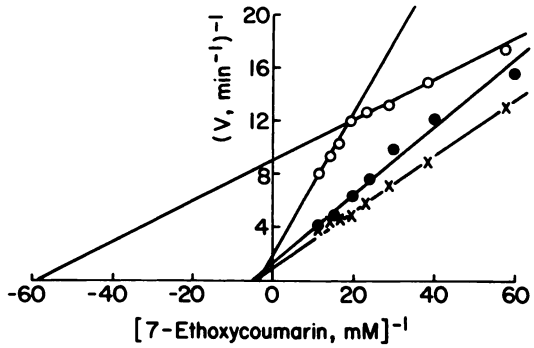


FIG. 5. *Lineweaver–Burk analysis of 7-ethoxycoumarin O-deethylation*

PB-adult microsomes (○); PB-neonate microsomes (●); and purified form 2 (×). Procedure is described under Methods.

heretofore unrecognized form of cytochrome P-450 or another protein could produce a pattern of peptide fragments similar to those of form 2,3,4 or 6. Yet the strong correlation exhibited between the peptide fragments obtained from purified form 2 and those fragments produced from the "induced" peptide occurring in PB-neonate microsomes, indicate that this microsomal component is most likely form 2. Further proof for this assignment is provided by immunological studies.

Monospecific antibodies produced against each of the four highly purified forms of cytochrome P-450 react selectively with specific binding sites on the respective protein antigen. The specificity manifested by these antibodies to the individual forms of cytochrome P-450 allows the identification of those forms as they occur in microsomal preparations. The presence of a positive reaction between the antibody to form 2 and PB-neonate microsomes indicates the presence of form 2, where the lack of reaction with untreated neonate microsomes connotes undetectable concentrations of form 2.

The synthesis of results obtained from the preceding complementary physical techniques denotes that following PB treatment, the cytochrome content of neonate microsomes is altered by the precocious appearance of form 2. Thus, we would expect the substrate selectivity of these microsomes to reflect the predominance of this form.

The metabolic form of an individual species of cytochrome P-450 as it exists in microsomes is usually difficult to assess. The coexistence of other forms and our present inability to ascertain the concentration of each of these forms in the microsomal matrix hinders the application of a form-specific analysis. However, if one form appears to predominate, as indicated by densitometric scans of SDS–PAGE, then the metabolic character of the microsomes will be governed to a greater extent by that form. When making comparisons between enzymatic activities of reconstituted forms of cytochrome P-450 and those of microsomes, we are interested in characteristic trends in metabolism. An example of such trend analysis is the high turnover of reconstituted form 2 supported metabolism of benzphetamine and the ability of PB-treated neonate microsomes to catalyze the N-demethylation of this substrate at rates greatly exceeding that observed for untreated neonate microsomes.

During the course of the reconstitution experiments, it was observed that a significant difference exists in the apparent $K_m$ values for 7-ethoxycoumarin among the various forms. Specifically, the apparent $K_m$ for form 2 is an order of magnitude greater than that of forms 4 and 6. Accordingly, the apparent $K_m$ for PB-neonate microsomes is in close agreement to that of form 2. The biphasic dependence on 7-ethoxycoumarin concentration evidenced by PB-adult microsomes, in which form 2 also predominates, is most likely the result of the contribution of another form(s) of cytochrome P-450 participating in the reaction, i.e., form 4 (Fig. 2).

The apparent absence of a form 2 entity in untreated neonate microsomes and subsequent appearance of this form in untreated adult microsomes indicate that the natural occurrence of this form is regulated by an age-dependent process. Temporal regulation of the appearance of multiple forms of cytochrome P-450 in microsomes has previously been established for both forms 4 and 6 (2). These forms are inducible by TCDD in adult rabbit hepatic tissues. Of these two forms, form 4 attains the greater increase in concentration. In the neonate, TCDD pretreatment results in the precocious appearance of only one form, form 6, as PB pretreatment effects the precocious appearance of form 2. Presently, the nature of the factors involved in this age-related control over the appearance of multiple forms of cytochrome P-450 is unknown.

As was shown in the Results section, the metabolism of xenobiotics by microsomes is reflective of individual forms of cytochrome P-450 participating in the reaction. In either adult or neonate rabbits, the response to a particular inducing agent can have profound consequences on the natural balance of xenobiotic metabolism. Since the individual forms of cytochrome P-450 are involved in both activation of injurious compounds as well as in their detoxification, either pathway may predominate as a result of induction, depending on the substrate under consideration.

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