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DIFFERENCES IN THE CONSTITUTIVE FORMS OF HEPATIC CYTOCHROME P-450 IN MALE AND FEMALE ADULT BEAGLE DOGS

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SUMMARY

Cytochrome P-450 isoenzymes were prepared from the solubilized liver microsomes of untreated adult male and female dogs, then separated into groups by high-performance liquid chromatography (HPLC). Partial purification was also completed through DEAE-52 cellulose and phosphocellulose ion-exchange chromatography. For comparison, solubilized hepatic cytochromes P-450 were obtained from rats dosed with phenobarbital (PB), β-naphthoflavone (BNF) or pregnenolone-16 α-carbonitrile. Minimal molecular masses of cytochrome P-450 subpopulations were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. HPLC and ion-exchange chromatography results suggested the presence of two or three major and several minor cytochrome P-450 subpopulations. Three distinct groups were predominant in the female and two major and two or three minor subpopulations were found in the male. One of two isoenzymes prominent in BNF-dosed rats was present in the male but was missing in the female dog; another minor canine cytochrome similar to one found in PB-dosed rats was missing from the male. These data indicate qualitative and quantitative sex-dependent differences in the constitutive cytochrome P-450 populations of the dog and suggest that HPLC analysis may be useful for the interpretation of toxicological studies where microsomal enzyme induction is suspected.

INTRODUCTION

The dog is frequently chosen as a suitable non-rodent species for safety evaluation studies of therapeutic agents or food additives targeted for human consumption. Occasionally, biological function tests such as antipyrine clearance time administered during the course of the toxicological studies or postmortem histopathology findings such as the proliferation of smooth endoplasmic reticulum (SER) suggest drug-related induction of drug-metabolizing enzymes in hepatic microsomes. Some examples of drugs that increase hepatic cytochrome P-450 levels or cause a proliferation of hepatic SER in the dog are phenobarbital

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(PB), phenylbutazone and clotrimazole [1,2]. Although highly purified cytochrome P-450 isoenzymes often have overlapping substrate specificity, the specific induction of particular cytochrome isoenzymes is characteristic of compounds such as β-napththoflavone (BNF) or PB [3,4].

As in other species, dose-related toxicological changes in experimental dogs observed after the administration of chemicals known to be metabolized by the liver may differ between males and females. These differences suggest fundamental differences either in the constitutive forms of hepatic cytochrome P-450 or the inducibility and/or susceptibility to inactivation of these cytochrome P-450 subpopulations depending upon the sex of the animal. The liver cytochrome P-450 composition of rats, mice, and rabbits [3] have been studied extensively in this regard, but few studies have been completed in the dog. A notable exception is the recent study of the hepatic drug-metabolizing enzymes of the beagle by McKillop [5], but that study did not include females. Recent advancements in the application of high-performance liquid chromatography (HPLC) for the analysis of rat cytochromes [6–8] suggest that HPLC analysis can also be used to identify the relatively low concentrations of individual cytochrome isoenzymes in liver microsomes from the normal dog. In this study, we used HPLC, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and analytical ion-exchange chromatography procedures for a comparative study of hepatic microsomal cytochrome P-450 subpopulations in male and female dogs.

EXPERIMENTAL

Animals

Pure-bred beagle dogs obtained from Marshall Farms (North Rose, NY, U.S.A.) weighed 8.1–11.6 kg each and were approximately one year old. Dogs were individually housed and fed Agway dog diet (Respond 2000®) supplemented with Big Red® dog food (Pro-Pet, Newark, DE, U.S.A.).

Male Long Evans rats weighing 200–300 g each were obtained from Charles River Breeding Lab. These were also individually housed and provided food and water ad libitum. Twenty-four animals were randomly assigned to one of four groups and treated as follows: (1) six were dosed with BNF (Sigma) in corn oil, 40 mg/kg for three days, intraperitoneally (i.p.); (2) six were dosed with PB (Merck) in 50 m*M* Tris-buffered 0.15 *M* potassium chloride, pH 7.4, 100 mg/kg for three days, i.p.; (3) six received pregnenolone-16 α-carbonitrile (PCN, Upjohn) in water and Tween 80, 50 mg/kg by oral gavage for five days; (4) six (controls) were dosed with 2 ml/kg Tris-buffered saline (see above) for three days, i.p. Following an overnight fast, rats were killed 24 h after the last dose.

Preparation of liver microsomes

Animals were euthanized with sodium pentobarbital. Rat livers were perfused in situ with cold 0.15 *M* potassium chloride in 50 m*M* Tris–HCl, pH 7.4. Dog liver lobes were first excised, then perfused through exposed veins. Livers were cut into pieces and homogenized with a Brinkman Polytron in 0.25 *M* sucrose buffered with 50 m*M* Tris–HCl, pH 7.4 (1 g per 3 ml). Homogenates were centrifuged

at 10 000 *g* for 20 min at 4 °C; then the supernatant was centrifuged at 100 000 *g* for 1 h at 4 °C to obtain microsomal pellets. Pellets were rehomogenized, recentrifuged once, and then stored at −60 °C. Protein content was determined by the biuret procedure [9] adapted for use on the Cobas-Bios centrifugal analyzer (Roche) using NERL (New England Reagent Laboratory, E. Providence, RI, U.S.A.) protein standards and Baker reagents.

Cytochrome P-450 content

Total cytochrome P-450 was determined by the method of Omura and Sato [10] as described by Guengerich [11]. Microsomes were resuspended in 0.1 *M* potassium phosphate buffer, pH 7.4, containing 1 m*M* EDTA, 20% glycerol, 0.5% (w/v) sodium cholate, and 0.4% (w/v) Triton N-101. Samples were divided into three equal parts. One aliquot was used for 400–500 nm baseline determination, the second sample was reduced with sodium dithionite and the net absorbance difference between the 450-nm peak and 490-nm baseline recorded, and the third sample was bubbled with carbon monoxide for 30 s, then reduced with sodium dithionite and the net 450–490 nm absorbance recorded. Total nmol cytochrome P-450 was calculated using the molar absorptivity of 91 mM^{-1} cm^{-1} [11] and corrected for total protein.

HPLC analysis of solubilized microsomes

Microsomal samples were prepared for HPLC analysis as described by Iversen and Franklin [8]. Microsomes were solubilized for 1 h at 4 °C in 10 m*M* sodium phosphate buffer (1:1, v/v) containing 0.75% (w/v) sodium cholate, 0.15 m*M* EDTA, 30% (v/v) glycerol and 0.3% (v/v) Emulgen 911 (Cometals, New York, NY, U.S.A.) all adjusted to pH 7.2.

HPLC analyses were carried out with a Waters (Millipore, Bedford, MA, U.S.A.) gradient system consisting of a Model 600 gradient module, a Model U6K/W universal injector, a Model 481 λ-max variable-wavelength detector, and a Model 740 data module integrator. A 100-μl sample was injected onto the Synchropak Anpac custom 100 AX 25×4.1 cm column (Anspec Warrenville, IL, U.S.A.; catalogue No. H1050) that was equilibrated with mobile phase buffer consisting of 20 m*M* Tris acetate, 0.2% (v/v) Emulgen 911, and 20% glycerol adjusted to pH 7.2. Elution with mobile phase buffer continued for 6 min at 1.6 ml/min after injection. This was followed by a 19-min linear gradient of 0–0.44 *M* sodium acetate prepared in the mobile phase buffer. At the completion of each analysis, the column was purged with 0.8 *M* sodium acetate dissolved in the mobile phase buffer. The absorbance of the eluate was monitored at 417 nm. Some cumulative fractions were collected and stored at −60 °C. These were later concentrated with an Amicon (Danvers, MA, U.S.A.) stirred cell ultrafiltration unit with a PM-30 membrane, then analyzed by SDS-PAGE.

Ion-exchange chromatography

Cytochrome P-450 purification was carried out by the procedure of Elshourbagy and Guzelian [12]. Microsomes were prepared from rat or dog livers as above except that after the second wash, these microsomes were suspended in a

minimal amount of buffer C (50 m*M* Tris–HCl, pH 7.9, containing 20% glycerol, 5 m*M* magnesium chloride, 0.1 m*M* EDTA, and 0.5 m*M* dithiothreitol). All steps were performed at 0–4°C unless indicated otherwise. Samples were suspended at a concentration of 7 mg total protein per ml of 50 m*M* potassium phosphate buffer, pH 7.4, containing 0.1 m*M* EDTA and 30% (v/v) glycerol. Sodium cholate was added dropwise with stirring to a final concentration of 3 mg per mg of protein. Solubilization was continued for 20 min.

A 50% solution of polyethylene glycol 8000 (PEG, Sigma) was added dropwise to a final concentration of 8%. After stirring for 20 min, the precipitate was removed by centrifugation at 36 000 *g* for 30 min and discarded. A 50% PEG solution was again added to a final concentration of 16%. After centrifugation at 100 000 *g* for 1 h, the supernatant was discarded and all pellets (except for the PCN rats) were resuspended in buffer A (10 m*M* potassium phosphate, pH 7.4, 20% glycerol, 0.1 m*M* EDTA, 0.2% Emulgen 911, and 0.5% sodium cholate). Pellets from PCN-dosed rats were resuspended in buffer B (20 m*M* Tris–HCl, pH 7.9, 20% glycerol, 0.1 m*M* EDTA, 0.5 m*M* dithiothreitol, 2.5 m*M* magnesium chloride, 0.1% Emulgen 911, and 0.25% sodium cholate). All were homogenized with a Brinkman Polytron homogenizer (Westbury, NY, U.S.A.).

DEAE-52 cellulose (Whatman, Hillsboro, OR, U.S.A.) was prepared according to the manufacturer's directions. The washed slurry was poured into a 40×2.6 cm Pharmacia column and equilibrated with buffer A except for PCN-dosed rat samples which required buffer B. All microsome samples (except PCN rat) were applied to a fresh column at 25°C, then eluted half-way down the column with 600 ml of 0.01 *M* buffer A at a flow-rate of 3 ml/min. Total protein and cytochrome P-450 load on these initial columns ranged from 318 to 806 mg protein and from 421 to 3169 nmol cytochrome, respectively. The dense red band containing cytochromes was removed, mixed with a minimal volume of buffer A to facilitate transfer, then applied to a second DEAE-52 column at 25°C equilibrated with buffer A. Separation of cytochromes from NADPH cytochrome P-450 reductase during the first chromatography step was complete when some parts of the column were tested for the latter. Cytochromes were eluted from the second column with a linear gradient of 0.02–0.12 *M* potassium chloride in buffer A. Flow-rates were either 3 or 4 ml/min and total gradient volumes were 500–600 ml. The elution of cytochromes was monitored at 417 nm. Potassium levels were determined by flame photometry. Column peaks were pooled, stirred gently for 2 h with Amberlite XAD-2 (Sigma), the resin removed, and the sample concentrated to 10 ml using the Amicon ultrafiltration cell.

Phosphocellulose (Whatman) prepared according to the manufacturer's directions was suspended in distilled water, poured into a 35×2.6 cm column (Pharmacia, Piscataway, NJ, U.S.A.) and equilibrated with buffer D (0.05 *M* potassium phosphate, pH 7.4, 0.1 m*M* EDTA, 25% glycerol, and 0.05% Emulgen 911). Samples were suspended in buffer D, applied to the column, and then 200 ml buffer D were passed through the column. Cytochromes were eluted with a linear gradient of 0.05–0.50 *M* potassium phosphate in buffer D. Flow-rates were 4 ml/min and total gradient volumes were 600 ml. Absorbance at 417 nm and K^+ were monitored as before.

Cytochromes from PCN-dosed rats were chromatographed as follows (all steps at 4 °C). Following PEG precipitation, microsomal protein was suspended in buffer B, stirred for 60 min, then applied to a DEAE-52 column equilibrated with buffer B. The column was washed with 1000 ml of 10 m*M* potassium chloride in buffer B. The cytochrome band was removed from the column as before, mixed with a minimal volume of buffer B, then loaded onto another DEAE-52 column, and eluted with a linear gradient of 0.02–0.20 *M* potassium chloride in buffer B. Pooled peak fractions were treated with XAD-2 and concentrated as above. This concentrate was diluted with three volumes of buffer C and applied to a 15×0.9 cm hydroxyapatite (Bio-Rad Labs., Richmond, CA, U.S.A.) column equilibrated with buffer C. The column was first washed with 100 ml of 0.02 *M* potassium phosphate, pH 7.4, containing 0.1 *M* EDTA, 0.2% Emulgen 911, and 0.5 m*M* dithiothreitol, and then eluted with an additional 0.1 *M* potassium phosphate, pH 7.4, in the above buffer. Fractions containing a single peak were pooled, then applied to a phosphocellulose column equilibrated with buffer E (0.02 *M* Tris–HCl, pH 7.4, 25% glycerol, 0.1 m*M* EDTA, 2.5 m*M* magnesium chloride, 0.5 m*M* dithiothreitol, and 0.05% Emulgen 911). The column was first washed with 500 ml of 0.05 *M* potassium chloride in buffer E then cytochromes were eluted with a linear gradient of 0.05–0.8 *M* potassium chloride buffer E.

Minimal molecular mass determination by SDS-PAGE

Solubilized microsomes and HPLC fractions were assayed by SDS-PAGE [13] to estimate the subunit molecular masses of cytochromes P-450 contained in those samples. The molecular standards, fumarase, glutamate dehydrogenase, and catalase from Boehringer Mannheim, were used as described by Wolf et al. [14]. Samples and standards were prepared for electrophoresis by dilution to a maximum of 1 mg protein per ml sample buffer (4 ml distilled water, 1 ml Tris–HCl, pH 6.8, 0.8 ml glycerol, 1.6 ml of 10% (w/v) SDS, 0.4 ml of 2-mercaptoethanol, and 0.2 ml of 0.05% (w/v) bromophenol blue) followed by heating to 95 °C for 4 min. Slab gels (0.75 mm×14 cm×14 cm) were prepared by combining 12% running gels with 4% stacking gels. The amount of total protein loaded was adjusted for each sample to optimize band resolution in the 48 000–60 000 relative molecular mass (M_r) area. Loaded gels were stacked at a constant voltage of 70 V (1–1.5 h) in a Bio-Rad Protean® vertical electrophoresis cell, then run at constant power (3 W, 4–4.5 h). Finished gels were fixed in isopropanol and acetic acid for 30–60 min minimum. These were then stained for ≥6 h in 0.1% Coomassie brilliant blue in water–methanol–acetic acid. To confirm the location of cytochrome protein, some gels were stained for heme by immersing in a 0.3 per mg/ml tetramethylbenzidine, ethanol, acetic acid solution followed by 3% hydrogen peroxide [15]. This revealed a diffuse blue–green staining hemoprotein region in the M_r 49 500–57 000 range with a faint, but distinct 52 000 band. Coomassie blue-stained gels were destained overnight in methanol–acetic acid. Gels were immersed in a glycerol–acetic acid solution for 45 min, then vacuum-dried between two sheets of dialysis membrane.

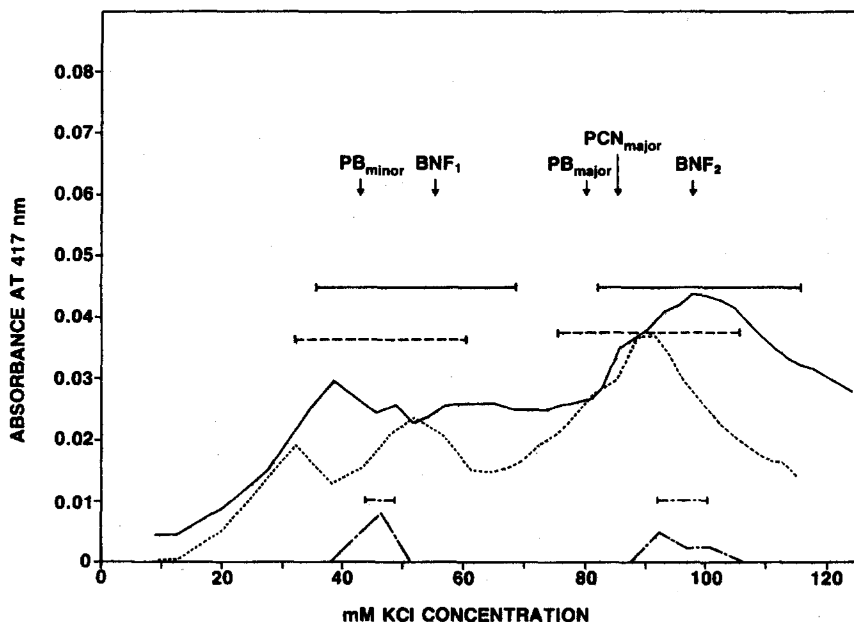

Fig. 1. Elution profiles from a DEAE-52 column for major cytochrome P-450 fractions isolated from male or female dog hepatic microsomes. The column was first equilibrated with buffer A (see text), then hemoprotein peaks absorbing at 417 nm were eluted with a linear gradient of 0.02–0.12 *M* potassium chloride. The location of major hepatic cytochrome P-450 peaks eluted from BNF-, PB-, or PCN-dosed rat microsomes (in separate experiments) are shown with arrows. Solid line, male dog; dotted line, female dog; mixed line, control rats. Horizontal bars indicate selection of fractions that were pooled.

RESULTS

Cytochrome P-450 content

Mean values (± 1 S.D.) for the hepatic cytochrome P-450 content of eleven dogs used for the HPLC work were 0.80 ± 0.37 nmol/mg of protein for the six males and 0.85 ± 0.75 nmol/mg of protein for the five females. The absorption maxima averaged 450 nm for each sex.

Ion-exchange chromatography

The eluate (Fig. 1) from the DEAE-52 column contained three distinct cytochrome P-450 peaks for the female and three less distinct peaks for the male as determined by absorbance at 417 nm. Eluate from these three general peak areas were pooled, concentrated, and rechromatographed on phosphocellulose. As shown in Fig. 2, three distinct cytochrome P-450 peaks were identified by absorbance at 417 nm in the column eluate for the female. These came off the column quite early in the gradient compared to the two measurable cytochrome peaks from the male. Four distinct cytochrome peaks for the control rat were not identical to any of the five different canine cytochrome peaks eluted from phosphocellulose. However, the major male dog cytochrome peak and the smallest female cytochrome

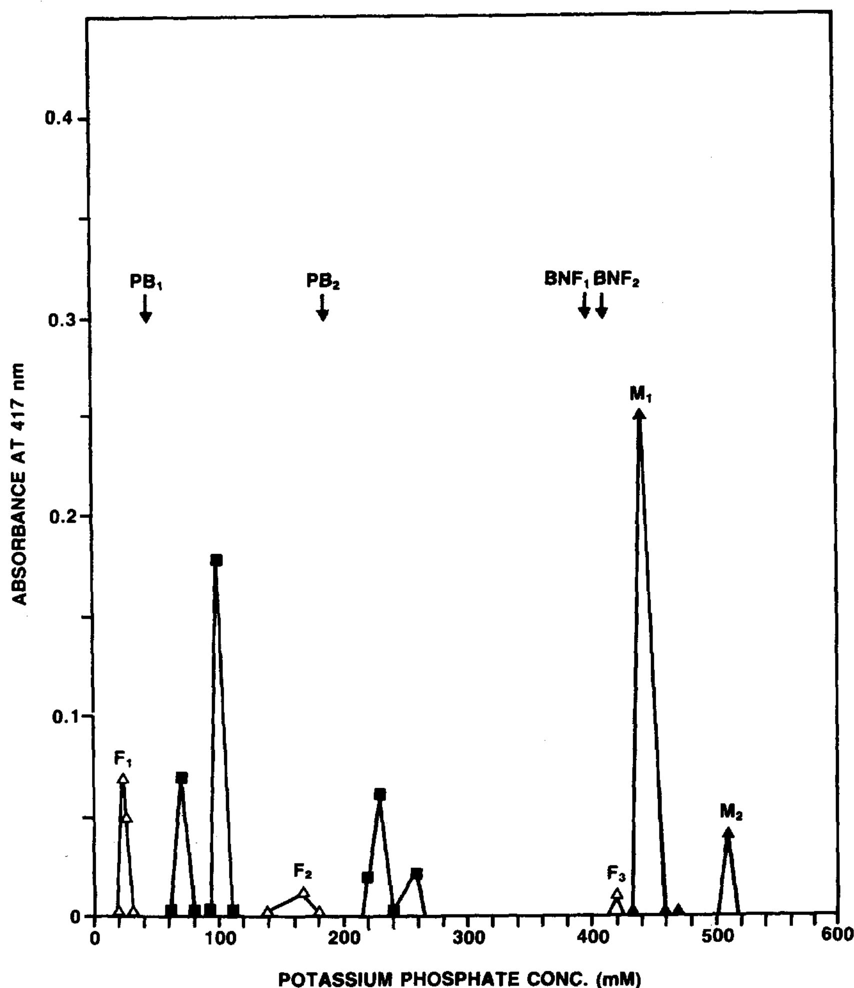

Fig. 2. Elution profiles from a phosphocellulose column for cytochrome P-450 fractions isolated from male or female dog microsomes and partially purified on DEAE-52 cellulose. The column was first equilibrated with buffer D (see text), then hemoprotein peaks absorbing at 417 nm eluted with a linear gradient of 0.05–0.5 *M* potassium phosphate in buffer D. The location of major hepatic cytochrome P-450 peaks eluted from PB- or BNF-dosed rat microsomes (in separate experiments) are shown with arrows. Symbols: △ = female dog; ▲ = male dogs; ■ = control rats. Peaks F_1–F_3 and M_1 and M_2 refer to the major hemoprotein peaks for female and male dog microsomes, respectively.

peak did elute at or near one of two major cytochrome peaks obtained from BNF-treated rats.

HPLC results

The HPLC profiles for PCN-, BNF-, PB-treated, or control rat hepatic cytochromes are shown in Fig. 3. Because of the presence of substantial NADPH cytochrome reductase or cytochrome b_5 [6,7], the retention time (t_R) 28–33 min peaks were omitted from consideration in these data. The specific cytochrome P-450 content of the rat hepatic microsome samples were: PB, 3.36 nmol/mg of

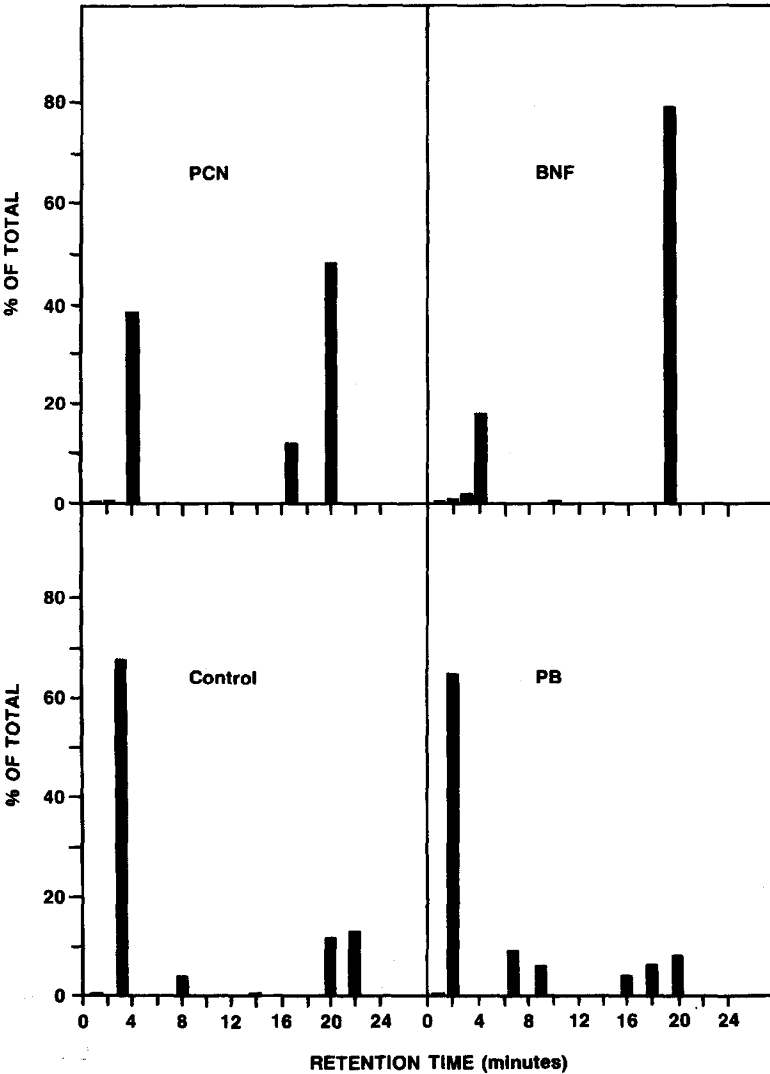

Fig. 3. Anion-exchange HPLC elution profiles of hemoprotein fractions absorbing at 417 nm obtained from the solubilized hepatic microsomes of PCN-, BNF-, PB-dosed, or control rats. Histobar height represents the percentage total peak area for all eluent (t_R=0–24 min) detected in each individual fraction. SDS-PAGE analyses of pooled HPLC fractions from the dosed rats suggested the following minimal molecular masses: PCN-dosed rates: t_R=17 min, M_r=51 000 and 53 000; BNF-dosed rats: t_R=19 min, M_r=54 000 and 58 000; PB-dosed rats: t_R=7 min, M_r=53 000; t_R=9 min, M_r=50 500; and t_R=16–18 min, M_r=50 000.

protein; BNF, 3.16 nmol/mg; PCN, 2.56 nmol/mg; and control, 1.7 nmol/mg. Compared to control cytochromes, PB treatment resulted in the appearance of cytochrome peaks at t_R=7, 9, 16, and 18 min; the PNC profile had a peak at t_R=17 min and a greatly increased peak at t_R=20 min, and BNF treatment produced a substantial peak at t_R=19 min.

Fig. 4 shows the collective results for the HPLC analysis of solubilized micro-

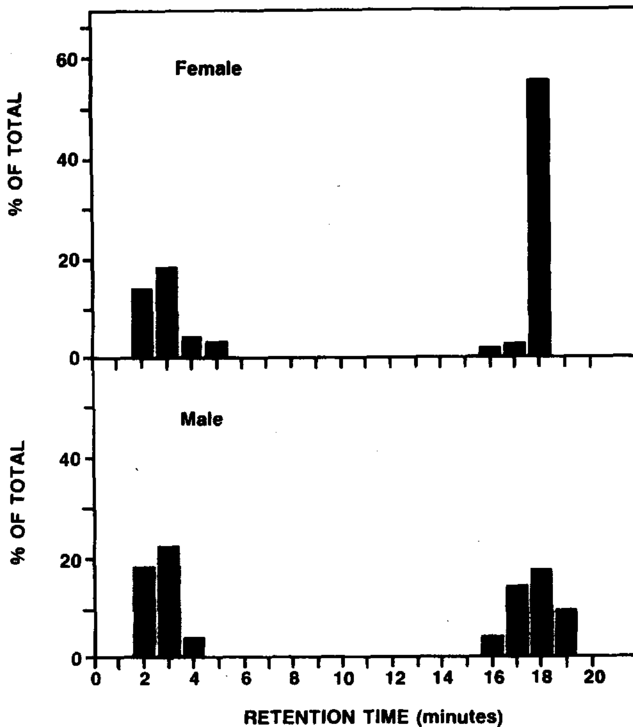

Fig. 4. Anion-exchange HPLC elution profiles of hemoprotein fractions absorbing at 417 nm obtained from the solubilized hepatic microsomes of male or female dogs. Each histobar represents the mean contribution to the total peak area for either six males of five females (eighteen independent HPLC assays on eleven samples) at the indicated retention time.

somes from the six male and five female dogs. Some major differences in the comparative HPLC profiles were as follows. For the female, the peak at $t_R = 18$ min constituted a substantial portion of the total cytochrome P-450 content and no peak was found at $t_R = 19$ min. For the male, there was negligible absorbance at $t_R = 5$ min, and the total cytochrome content as measured by absorbance at 417 nm was more uniformly distributed among the peaks at $t_R = 16$–18 min than in the female.

When HPLC eluate was collected, concentrated by ultrafiltration, then electrophoresed according to minimal molecular weight on SDS-PAGE, the results shown in Table I were obtained. These data indicate that the two to three major peaks detected during the isocratic phase (i.e., $t_R = 0$–6 min) of the HPLC elution contain all the major canine cytochromes while peaks detected during the gradient elution phase contain one or two individual canine cytochrome species of low, intermediate, or high molecular mass.

SDS-PAGE results and relevance to other data

Minimal molecular mass estimates ($\pm 2\%$) for the major cytochrome composition of solubilized microsomes from the four rat groups and pooled results for several analyses of solubilized canine microsomes are shown in Table II. In the

TABLE I

MINIMAL MOLECULAR MASSES (M_r) OF CYTOCHROME P-450 ISOENZYMES IN HPLC FRACTIONS FROM SOLUBILIZED CANINE MICROSOMES AS DETERMINED BY SDS-PAGE

Minor bands are shown in parentheses.

Fraction No.	t_R range (min)	M_r of cytochrome P-450
I	0 - 2.5	
II	2.5- 7	(48 500), 50 500*, 51 500, 52 000, 54 000*, and 55 500*
III	7 -10	51 000, 52 500
IV	15 -19	52 500, 54 000
V	19 -25	52 000, 55 000
VI	31 -35	67 000

*Major band.

PB-treated rat, the major cytochrome band was very broad, centered around $M_r = 52\,000$. This single or multiple cytochrome fraction is represented by the HPLC fractions at $t_R = 7$ and 9 min in Fig. 3 and by one or both PB peaks indicated by arrows in Fig. 1. The cytochromes P-450 from BNF-treated rats represented by the $M_r = 53\,000$ and 58 000 bands are similar if not identical to the hemoproteins eluting as the HPLC peak at $t_R = 19$ min, and to the two BNF peaks indicated by arrows in Fig. 2. HPLC results suggest that PCN treatment increased the cytochrome group at $t_R = 20$ min found in the control rats and gave rise to a

TABLE II

MINIMAL MOLECULAR MASSES (M_r) OF MAJOR CYTOCHROME P-450 ISOENZYMES FOUND IN SOLUBILIZED RAT AND CANINE HEPATIC MICROSOMES AS DETERMINED BY SDS-PAGE

Values shown are ±2% (i.e., ±1 S.D.). PB rat cytochrome $M_r = 52\,000$, BNF rat cytochrome $M_r = 54\,000$, and PCN rat cytochrome $M_r = 51\,000$ correspond to major SDS-PAGE bands reported by Ryan et al. [16] for P450b and by Elshourbagy and Guzelian [12] for 3-methylcholanthrene- and PCN-treated rats, respectively.

PB rat	BNF rat	PCN rat	Control rat	Female dog	Male dog
49 000		49 000		49 000*	49 000*
50 000*	50 000		50 000		
		51 000		51 000*	51 000*
52 000*		52 000	52 500*		
	53 000*				53 000*
		54 000*		54 000	
			55 000		55 000
56 000	56 000				
		57 000		57 000	
	58 000		58 000		58 000

*Major band.

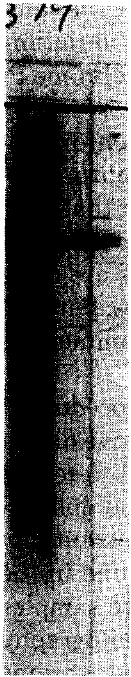

Fig. 5. SDS-PAGE analyses of solubilized hepatic microsomes from a male beagle dog (first lane) versus a representative HPLC fractions from that same animal.

new HPLC hemoprotein group at $t_R = 17$ min with approximate minimal molecular masses of $M_r = 51\,000$ and/or 53 000.

On SDS-PAGE, the solubilized microsomes from both the male and female dog yielded three major cytochrome bands with similar, but not necessarily identical molecular masses. The male $M_r = 58\,000$ cytochrome may be the same as the HPLC group at $t_R = 19$ min (Fig. 4). Although the SDS-PAGE, DEAE-52, and phosphocellulose chromatography results all indicate three major cytochrome groups in the female, the female microsomes lack the cytochrome subgroup at $t_R = 19$ min assayed by HPLC. A comparison of SDS-PAGE results for solubilized microsomes from a male dog versus a representative HPLC fraction from the dog is illustrated in Fig. 5.

DISCUSSION

The cytochrome P-450 content of solubilized microsomes from the male beagle dog following treatment of dogs with phenobarbitone or BNF have been reported recently by McKillop [5]. Using the protein profiles on SDS-PAGE, he described three major protein bands with $M_r = 49\,500$, 50 500 and 52 000. An additional constitutive $M_r = 58\,000$ cytochrome was found that was inducible in both rats and dogs, but that study did not include the female beagle. While SDS-PAGE analysis provides an extremely useful method for the identification and characterization of cytochromes P-450, not all proteins in the $M_r = 48\,000$–$60\,000$ range

are cytochrome P-450, and more than one specific cytochrome can have the same SDS-PAGE subunit molecular mass [16]. Also, due to experimental variation, different subunit molecular masses have been reported for a given cytochrome P-450, even within the same laboratory [3].

In this study, we utilized both HPLC and classical ion-exchange chromatographic procedures for the isolation and identification of major cytochrome P-450 subpopulations in the male and female beagle dog. Our data suggest that even though both the male and female dog have the same nominal number of major cytochromes P-450 when assayed by SDS-PAGE, these major groups are quite different when compared by ion-exchange chromatography procedures, particularly after the DEAE-52 and subsequent phosphocellulose steps recommended for cytochrome purification [12].

Concurrent analysis of solubilized dog microsomes via the HPLC procedure of Iversen and Franklin [8] demonstrated both qualitative and quantitative differences between the two microsomal sources. In particular, quantitative differences were noted in the fractions at $t_R = 17$–18 min ($M_r = 52\,500$–$54\,000$) and qualitative differences were obvious in the fraction at $t_R = 5$ min, which was essentially absent in the males, and the fraction at $t_R = 19$ min, which was not found in the female microsome fractions. SDS-PAGE analysis of the HPLC eluate samples from this range and comparative studies with phosphocellulose chromatography suggest that this latter fraction is quite similar to one of the major cytochrome P-450 groups that is elevated by BNF in the rat. The identity of the peak at $t_R = 5$ min found primarily in the female is less clear, although this peak is closest to the cluster of PB-inducible cytochrome peaks in the HPLC profile for rat cytochromes. SDS-PAGE results and data from the phosphocellulose chromatography step further suggest some similarity between this female dog cytochrome and one of two major PB-inducible cytochrome P-450 species in the rat.

In summary, some major differences were found in the biochemical characteristics of cytochrome P-450 isolated from male and female dog livers. These canine cytochromes were generally dissimilar to constitutive cytochrome P-450 species isolated from the male rat liver although a cytochrome found in the male dog liver was quite similar to a BNF-inducible cytochrome of the male rat and a cytochrome found in the female dog liver shared some similarities with a major PB-inducible cytochrome P-450 of the male rat. These data suggest that HPLC analysis can be used for the rapid detection of altered cytochrome P-450 in toxicological studies in male and female dogs.

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