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Cytochrome P-450 and Monooxygenase Activity in Cardiac Microsomes from the Fish *Stenotomus chrysops*

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SUMMARY

Optical spectroscopy of dithionite or NADPH-reduced, CO-treated microsomes (having little mitochondrial contamination) indicated the presence of cytochrome P-450 with an absorption maximum near 447 nm in both ventricle and atrium from the heart of the marine fish scup (*Stenotomus chrysops*). Furthermore, low-temperature (approximately 14° K) EPR spectra of microsomes from ventricle and atrium had resonances with *g* values near 2.42, 2.26 and 1.91, typical of low-spin cytochromes P-450. Average values for the specific P-450 content of these microsomes were 0.18 nmole/mg in ventricle and 0.25 nmole/mg in atrium, but large variation was observed. In contrast to muscular parts of the heart, the specific P-450 content in bulbus arteriosus (aorta) microsomes was very low (0.003 nmole/mg), and the wavelength of the reduced-CO absorption maximum was like that in liver microsomes. The levels of CO-inhibitable benzo[*a*]pyrene (BP) monooxygenase, 7-ethoxyresorufin *O*-deethylase and aminopyrine *N*-demethylase activities per nanomole of P-450 were 10–30 times lower in cardiac microsomes than in liver microsomes. Treatment with 3-methylcholanthrene produced an insignificant change in BP monooxygenase activity, yet the metabolism of BP was strongly inhibited by 7,8-benzoflavone in cardiac microsomes from either control or treated animals. Microsomes from both ventricle and atrium metabolized BP with pronounced regiospecificity. More than 50% of the total metabolites were 7,8- and 9,10-(benzo-ring) dihydrodiols, while 4,5-(K-region) dihydrodiol was undetectable. Whether the low catalytic activities with the xenobiotics seen here are associated with major or minor (constitutive or induced) forms of the cardiac microsomal cytochrome P-450 is not yet known, but the results are consistent with the idea that other, undefined, functions may be the principal ones of this hemoprotein in microsomes from fish and possibly mammalian heart.

INTRODUCTION

Cytochromes P-450[5] catalyze monooxygenase reactions important in the biotransformation of numerous foreign compounds and in the metabolism of steroids (1) and other endogenous compounds such as vitamin D (2). Abundant evidence has now confirmed the existence of multiple forms of P-450 which may explain differences in monooxygenase activities in different tissues, sexes, and developmental stages and in animals treated with different xenobiotics (e.g., ref. 3). Monooxygenase systems are best known in liver, where adaptive functions in foreign compound metabolism are prominent. Such adaptive functions may also be important in other tissues proximate to the environment, such as mammalian lung (4). Other functions of P-450 are prominent in some internal organs, such as occur during steroidogenesis in mitochondria of the adrenal cortex (5, 6) and during vitamin D

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[5] The abbreviations used are: P-450, cytochrome P-450; BP, benzo[*a*]pyrene; 7,8-BF, 7,8-benzoflavone (α-naphthoflavone); AP, aminopyrine; TLC, thin-layer chromatography; HPLC, high-pressure liquid chromatography; ER, ethoxyresorufin; 3-MC, 3-methylcholanthrene.

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hydroxylation in kidney mitochondria (2). P-450 and monooxygenase activities have been detected in most organs of vertebrates, but the nature of these systems and their roles in metabolism of endogenous as opposed to foreign compounds are in many cases still completely unknown.

Earlier reports of monooxygenase activity in heart did not disclose the presence of P-450, but recently a high specific content of P-450 was seen in heart of the marine fish scup (7) and of Sprague-Dawley rats (8). In both studies a survey of electron transfer components in "microsomes" from various tissues revealed that the specific content of P-450 in fractions prepared from heart were second only to those in liver. Moreover, in the scup the Soret maximum reported for reduced, CO-ligated P-450 in heart (about 447 nm) was distinct from that observed in other tissues (7). However, in neither the fish nor the rat was the possible contamination of the microsomal preparations by other chromophores fully described. Furthermore, neither the complete subcellular nor the intra-organ localizations were determined, and the catalytic functions possibly associated with cardiac P-450 were only briefly described. In this communication we provide conclusive evidence for the occurrence of P-450 in cardiac tissue microsomes, describing the intra-organ distribution and some characteristics of P-450 in scup heart atrium and ventricle. We also report the patterns of metabolism *in vitro* of the environmental carcinogen BP by scup cardiac microsomes.

MATERIALS AND METHODS

Chemicals. BP (Gold Label), 7,8-BF, and 1,1,1-trichloropropene oxide were obtained from Aldrich Chemical Company (Milwaukee, Wisc.). AP was obtained from Aldrich Chemical Company and recrystallized from methanol. [^{3}H]BP was obtained from Amersham/Searle (Skokie, Ill.). Resorufin was obtained from MCB Chemical Company (Cincinnati, Ohio) and was purified as follows: 1 g of resorufin was dissolved in a solution of 100 ml of dimethylformamide, 20 ml of pyridine, and 20 ml of acetic anhydride. After 12 hr at room temperature the reaction mixture was dried to reduce volume, extracted with chloroform, washed with 0.1 M $NaHCO_3$, and evaporated to dryness. The product was dissolved in a small volume of $CHCl_3$-CH_3CN (9:1) and chromatographed on silica gel thin-layer plates with the same solvent system as mobile phase. Orange fractions with an R_F of 0.25 were pooled, extracted from the silica, dried, and recrystallized from acetone. This material (acetylresorufin) had a melting point of 229–229.5° and was judged pure by 90 mHz NMR, elemental analysis, and TLC. Resorufin was obtained by dissolving acetylresorufin in methanol, hydrolyzing the ester with 20 mM NaOH for 15 min at room temperature, and precipitating the resorufin by the addition of HCl to pH 1. The precipitate was recrystallized from hot DMF. These crystals were free of Cl^- by negative Beilstein test and judged pure by 250 mHz NMR, TLC, HPLC, and elemental analysis. ER was synthesized according to the methods of Prough *et al.* (9), and was judged pure by TLC and NMR. NADP, NADPH, NADH, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, and horse heart cytochrome *c* were obtained from the Sigma Chemical Company (St. Louis, Mo.). Sodium succinate was obtained from Fisher Scientific Company (Medford, Mass.). Materials for HPLC were obtained as before (10).

Animals. Scup (*Stenotomus chrysops*, formerly called *S. versicolor*), ranging between 100–600 g, were obtained in local Woods Hole waters between May 1979 and August 1980. Some animals were used immediately after capture whereas others were held for periods of up to 7 months in 800-gallon aquaria equipped with flowing seawater at 20° ± 1°. Animals held were maintained on a diet of Purina trout chow and chopped smelt. Most animals were used untreated but one group received i.p. injections of 3-MC (15 mg/kg) in corn oil and were killed 24 hr later. Phenobarbital was not selected as it is ineffective as an inducer in fish liver.

Tissue preparation. All procedures were carried out at ice temperature. Heart and liver were removed immediately from the animals and rinsed with ice-cold buffer. Ventricle, atrium, and the vascular bulbus arteriosus were dissected and weighed. Ventricle plus atrium equaled 0.15 ± 0.03% of body weight. Tissues were finely minced and homogenized in 10 volumes of 50 mM Tris or 50 mM potassium phosphate (pH 7.4) containing 1.15% KCl. Samples were homogenized with a Potter-Elvehjem tissue grinder with four passes at 1900 rpm and four at 2750 rpm. Subcellular fractionation was accomplished as previously described (7). Nuclear, mitochondrial, and microsomal fractions were resuspended in 50 mM Tris containing 1 mM EDTA, 1 mM dithiothreitol, and 20% glycerol by volume. Resuspension was in 1–3 volumes of buffer per gram of tissue (wet weight). Protein content of fractions ranged from 10 to 20 mg/ml (nuclear), 9 to 12 mg/ml (mitochondrial), 2 to 14 mg/ml (microsomal), and 4 to 11 mg/ml (supernatant). Preparations were used directly or were occasionally stored in liquid N_2 until use. Blood samples were drawn from the caudal vein of some fish, and the red cells were pelleted by centrifugation. Pellets were resuspended in 1 volume of buffer and used either directly or after lysis.

Electron microscopy. Freshly prepared microsomal pellets were fixed directly for 6 hr at 5° with a solution that was 3% glutaraldehyde, 1.5% formaldehyde (generated from paraformaldehyde), 0.05% $CaCl_2$, and 5% sucrose in 0.1 M cacodylate buffer (pH 7.4). Pellets were postfixed with osmium tetroxide and embedded in Spurrs resin; thin sections were examined with a Hitachi HS-9 electron microscope.

Spectroscopy. Cytochrome P-450 was assayed by $Na_2S_2O_4$ difference spectra of CO-treated samples as previously described (11), with resuspensions containing about 1 mg of microsomal protein per milliliter. CO-treated microsomes were also reduced with NADPH, at 0.34 mg/ml. Assays for cytochrome b_5 were carried out with similar dilutions and NADH as reductant as previously described (11). Spectral analyses of heart mitochondrial preparations were carried out with resuspensions containing about 1 mg of mitochondrial protein per milliliter. Heart mitochondria and liver microsomes mixed in resuspensions that contained about 0.5 mg of protein from each were assayed as above. In some assays of each type of preparation, succinate and KCN were

added to achieve final concentrations of 5 mM and 1 mM, respectively, in both sample and reference cuvettes. Microsomal pellets for EPR were frozen using an isopentane bath at approximately 120° K so that freezing was complete within 3 sec. EPR spectra were recorded at 14° K with a Varian E-9 spectrometer with microwave frequency approximately 9.05 gHz, microwave power 0.3 mW; modulation frequency 100 kHz, modulation amplitude 10 G, scanning rate 250 G/min, time constant 0.3 sec, field center 2960 G, and scan range 1000 G.

Enzyme assays. BP monooxygenase activity was assessed by determining the production of fluorescent derivatives or by measurement of the total production of water-soluble derivatives of [^{3}H]BP. The former was accomplished as previously described (7). The latter was accomplished by a modification of the radiometric procedure of Van Cantfort *et al.* (12). The reaction mixture consisted of 0.05 M Tris-HCl with bovine serum albumin (1.4 mg/ml), 0.40 mM NADPH or NADH, 70 μM [^{3}H]BP (approximately 150 μCi/μmole), and about 60 μg of sample protein in a volume of 50 μl. The reaction was initiated with 2 μl of [^{3}H]BP in methanol, after which the samples were incubated for 15 min at 29°. The temperature optimum for BP monooxygenase activity in both atrium and ventricle was determined to be near 30°. Activity in both was maximal at pH between 7.0 and 7.4. Reactions were stopped with 100 μl 0.15 M KOH in 85% dimethyl sulfoxide and were extracted three times with 0.5 ml of hexane. A portion (50 μl) of the aqueous layer containing the metabolites was added to 3 ml of Aquasol (New England Nuclear acidified with 30 μl 0.6 N HCl, and counted in a Beckman LS-100C liquid scintillation counter. Counting efficiency was determined with internal standards. The influence of 7,8-BF on BP metabolism was determined by adding 2 μl of 7,8-BF in methanol to the BP monooxygenase reaction mixture just prior to addition of [^{3}H]BP. BP metabolism in both ventricular and atrial microsomes was linear with time to 20 min and with protein to 0.15 mg/ml of reaction mixture, and assays were carried out in the linear range. Blanks consisted of reaction mixtures minus cofactor. Assays were carried out in triplicate under red light.

Ethoxyresorufin O-deethylase activity was measured using a 1.0 ml of reaction mixture containing 2 μM 7-ER, 0.1 M Tris (pH 8.0), 0.1 M NaCl, and microsomes (22 μl) (3–8 mg of protein per milliliter). The reaction at 25° was initiated with NADPH at a final concentration of 0.5 mM and the appearance of resorufin was monitored at 572 nm using a Cary 118C recording spectrophotometer. The extinction coefficient of resorufin was experimentally determined to be 73 mM^{-1} cm^{-1}.

Aminopyrine demethylase activity was assayed by measuring formaldehyde generated in reaction mixtures described previously (7). Reactions were stopped after 15 min and formaldehyde was determined as before (7). The pH optimum determined for cardiac AP demethylase was broad and the temperature optimum was 35°, but reactions were routinely incubated at 30°.

Cytochrome *c* reductase activities were assayed at 25° as before (7). NADPH-dependent activity was assayed with a reaction mixture containing 0.175 mM NADPH, 80 μM horse heart cytochrome *c*, and 1 mM KCN in 0.2 M potassium phosphate buffer (pH 7.7). NADH-cytochrome *c* reductase activity was assayed using the conditions for NADPH-cytochrome *c* reductase, with 0.25 mM NADH replacing 0.175 mM NADPH. Succinate-cytochrome *c* reductase activity was assayed in reaction mixtures of 0.77-ml volume containing bovine serum albumin (10 mg/ml), 80 μM cytochrome *c*, and 1 mM KCN in 0.02 M phosphate buffer (pH 7.4). Reactions were initiated by addition of sodium succinate to 5 mM. Reduction of cytochrome *c* at 550 nm was followed using a Cary 118C recording spectrophotometer.

Practical limits of detection determined empirically for these assays under the conditions used were as follows: cytochrome P-450, 0.01 nmole/mg of microsomal protein; cytochrome b_5, 0.005 nmole/mg; cytochrome *c* reductases, 0.2 nmole of cytochrome *c* reduced per minute per milligram; AP demethylase, 0.13 nmole of formaldehyde produced per minute per milligram; 7-ER *O*-deethylase, 0.015 nmole of resorufin produced per minute per milligram; BP monooxygenase measured radiometrically, 1.0 pmole per minute per milligram; BP monooxygenase measured fluorometrically, 0.5 pmole of 3-OH-BP equivalents produced per minute per milligram. Extraction efficiency for 3-OH-BP in this assay was 50%, and the data have been corrected for this. Protein was determined according to the method of Lowry *et al.* (13).

HPLC. Metabolites of [^{3}H]BP were obtained *in vitro* with microsomes prepared from pooled scup atria and ventricles. Reaction mixtures (1 ml) containing 60 μM BP, NADPH (0.375 mg/ml), and 2.0–5.9 mg of microsomal protein per milliliter were incubated for 20 min and the metabolites were extracted and prepared as before (10). Zero-time and boiled-enzyme blanks were treated in a similar fashion. Separation of BP metabolites was achieved using a DuPont LC 850 chromatograph fitted with a 50-μl injection loop, a 25-cm DuPont Zorbax ODS column, and filter photometer UV detector operating at 254 nm. Metabolites were eluted by running a gradient from 40–85% acetonitrile in water. Metabolites were identified on the basis of co-elution with authentic standards and quantified by counting the labeled fractions eluting from the column. All procedures were carried out under red light. Recovery was determined for each metabolite by isolating labeled metabolites previously produced and carrying these individually through the complete procedure used for zero-time blanks. Recoveries ranged between 61 and 83%, with a mean at about 70%. Data on BP metabolites were corrected for the specific recoveries.

RESULTS

The data in Fig. 1 indicate that succinate-cytochrome *c* reductase activity and NADH-cytochrome *c* reductase activity of scup ventricle, atrium, and bulbus arteriosus are localized in a mitochondrial fraction prepared by differential centrifugation, whereas NADPH-cytochrome *c* reductase and BP-monooxygenase activities are localized in the microsomal fraction prepared from these tissues. The structural characteristics of the microsomal fraction of ventricle visualized with the electron microscope (Fig. 2) are typical of microsomal fractions prepared from other tissues. Such structures may well be derived from the sarcoplasmic reticulum of fish cardiac

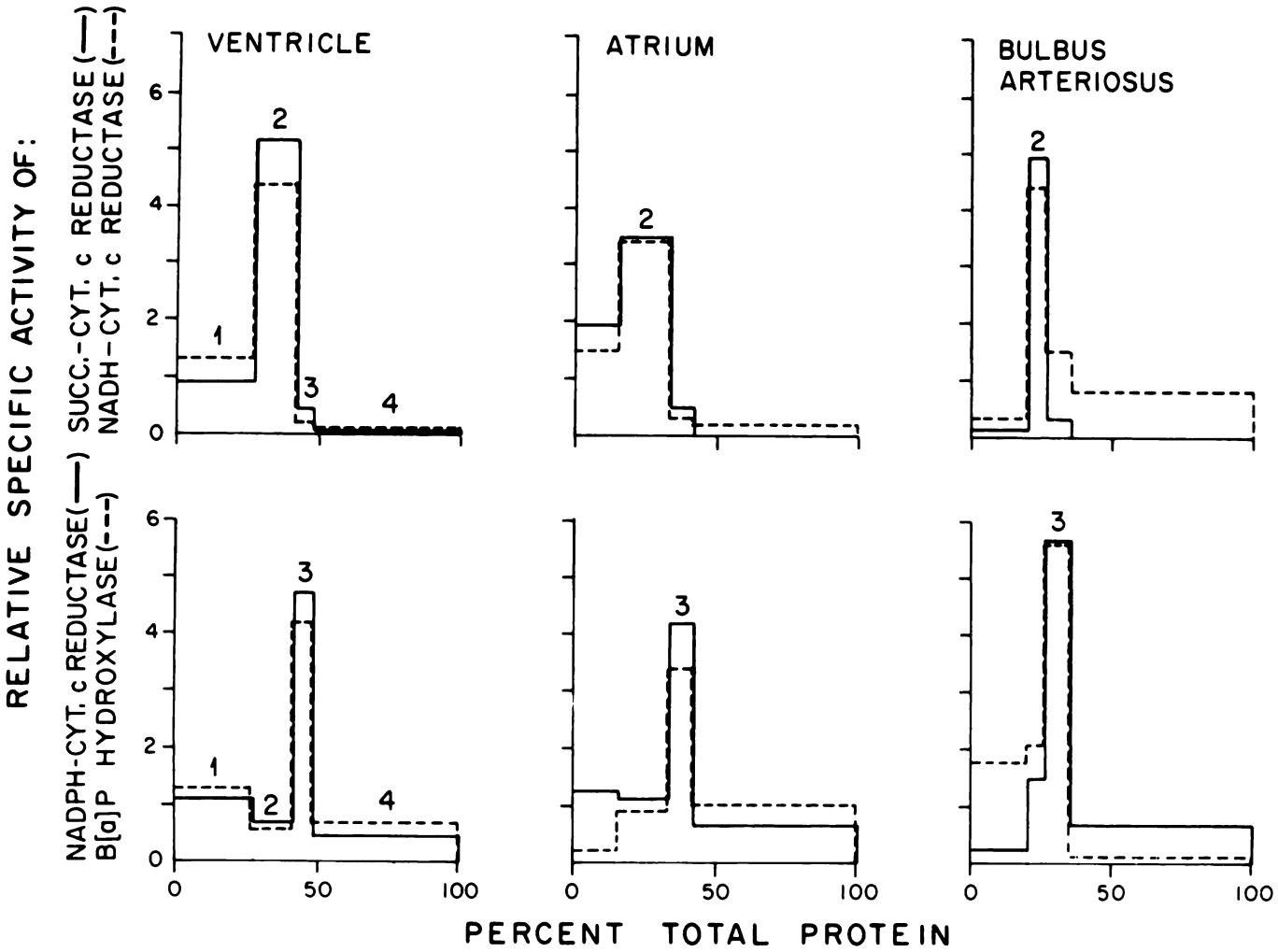

FIG. 1. *Subcellular distribution of cytochrome c reductase activities and BP monooxygenase activities in cardiac tissues of Stenotomus chrysops*

Relative specific activities were calculated according to DeDuve *et al.* (14). *1*, Nuclear; *2*, mitochondrial; *3*, microsomal; and *4*, cytosolic fractions. Data represent results of three separate fractionations, or, in the case of bulbus, two fractionations. NADPH-dependent reductase activity in nuclear fractions of ventricle and bulbus were determined from a single fractionation.

cells. Microsomal preparations of tissues rich in mitochondria, such as heart, can easily be contaminated by mitochrondrial fragments that could influence the results obtained. Although not shown in Fig. 2, electron microscopic examination revealed the presence of mitochondrial fragments in some microsomal preparations of scup heart. That contamination was very limited is shown by the data in Table 1, which lists the total cytochrome *c* reductase activities in mitochondrial or microsomal preparations, normalized per gram of tissue. Less than 2% of the total succinate-dependent reductase activity appeared in the microsomal fractions of heart, about the same as in the liver. The percentage of NADH-cytochrome *c* reductase activity in heart microsomes differed very little from that seen with succinate, whereas in liver 75% or more of the NADH-dependent activity was seen in microsomes. However, the major portion of NADPH-dependent activity was present in microsomes in each case.

Consistent with earlier observations (7), the Soret maximum of dithionite reduced, CO-treated microsomes from atrium and ventricle was at about 447 nm. Although mitochondrial contamination was generally low in heart microsomes, interference in spectrophotometric assays was still possible, since the level of microsomal NADPH-cytochrome *c* reductase activity was very low as well. However, there was no evidence of spectral interference by mitochondrial cytochrome oxidase (a–a_3) in some samples; i.e., there was no peak at 430 nm nor a deep trough at 424 nm in the dithionite difference spectrum of CO-treated microsomes (e.g., Fig. 3A). Moreover, the peak near 447 nm was noticeably absent in scup heart mitochondria under the same conditions. Elimination of the cytochrome oxidase signal present as a "shoulder" at 443–445 in reduced, CO-treated scup heart mitochondria could be achieved by addition of succinate and CN to both sample and reference. Heart microsomes so treated still exhibited a peak at 447 nm. We further observed that the Soret maximum of a sample of scup liver microsomes (450 nm) assayed in a 1:1 mixture with scup heart mitochondria was not shifted when succinate and CN were present in the cuvettes, lending confidence to our observation of a 447-nm absorption maximum for scup heart microsomes. Furthermore, this was strongly sup-

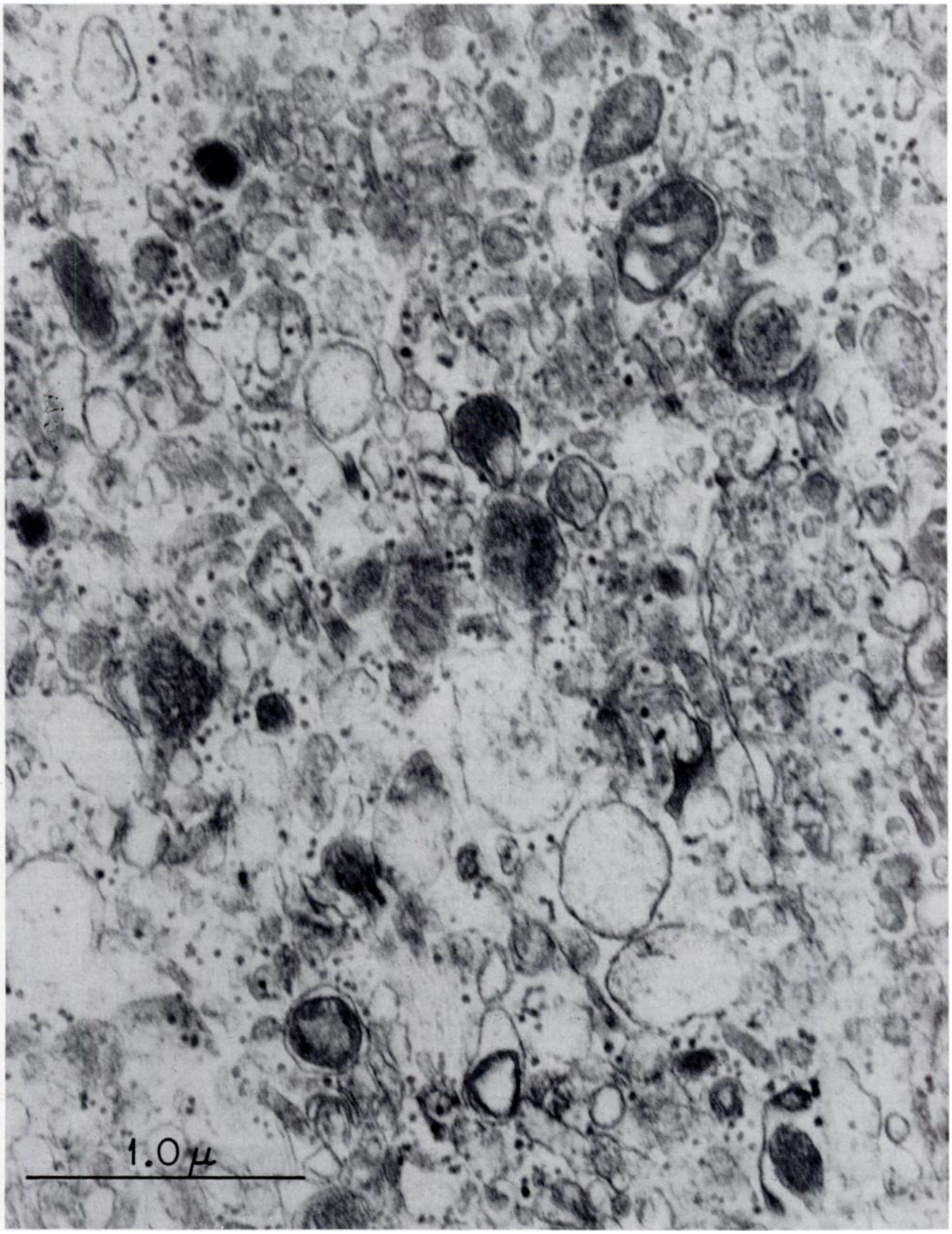

FIG. 2. *Electron micrograph of Stenotomus chrysops ventricular microsomes*
Original magnification was × 20,000.

TABLE 1

Distribution of cytochrome c reductase activities in Stenotomus chrysops

Total activity in each fraction was normalized to 1 g of tissue.

Preparation	Activity dependent on		
	Succinate	NADH	NADPH
	nmoles cytochrome c reduced/min/g tissue		
Ventricle			
Mitochondria	2478	3761	8
Microsomes	52	88	22
Atrium			
Mitochondria	3323	2523	41
Microsomes	66	67	93
Liver			
Mitochondria	1021	793	291
Microsomes	19	2555	1463

ported by the appearance of a peak near 447 nm in heart microsomes that had been bubbled with CO and then reduced with NADPH (Fig. 3B).

The conclusion that these spectrophotometric observations of heart tissues indicate the presence of P-450 was confirmed by low-temperature (~14° K) EPR spectra of both ventricle and atrium microsomes (Fig. 3C). In both cases the spectra obtained showed resonances with *g* values (Table 2) that are typical of low-spin cytochromes P-450 (15). The *g* values for ventricle and atrium differed only slightly from those seen with liver microsomes, and there was no apparent high-spin P-450 in the heart samples. Comparison of the normalized peak height of the resonance at each of the three *g* values for a sample of heart P-450 with those obtained for a known amount of scup liver P-450 confirmed that the specific content of heart P-450 based on optical spectra was approximately correct, to within ±15%.

The levels of P-450 were appreciable in both ventricle and atrium microsomes (Table 3). This condition in the muscular parts of the heart is clearly distinct from that in the bulbus arteriosus, where the specific content was quite low. The absorption maximum of bulbus microsomes was about 449.5 nm, quite like that in liver. The levels of atrial and ventricular P-450 given in Table 3 are like those we usually see in these tissues, but occasional samples of heart have had levels as low as 0.05 nmole/mg or as high as 0.91 nmole/mg of microsomal protein. In the latter case the specific content in heart was almost twice that in liver microsomes from the same animals. However, the yield of cardiac P-450 normalized to tissue or body weight ranged between 0.8 and 1.2 nmoles/g of tissue, or 0.001 nmole/g of body weight, substantially less than the 7.5 nmoles/g of tissue, or 0.08 nmole/g of body weight, for hepatic P-450. Cytochrome b_5 could not be detected in any of the heart samples.

In contrast to the specific content of P-450, the specific activity of NADPH-cytochrome *c* reductase in ventricle or atrium microsomes was only 4–7% that in liver (Table 3). The levels of monooxygenase activity were also very low in heart tissue microsomes (Table 3). The requirements for BP metabolism (not shown) were typical of a monooxygenase activity; i.e., NADPH and O_2 were required and the activity was inhibited by CO. The activity was supported by NADH at only about 10% of the rate seen with NADPH. The activity seen was apparently not associated with hemoglobin (16) present as a contaminant in the heart microsomes. Using the same conditions we could not detect any turnover of BP by either whole or lysed scup red cells. Estimated turnover numbers for BP metabolism in both atrium and ventricle were about 0.1 nmole/min per nanomole of P-450 as compared with 2.0 nmoles of 3-OH-BP Eq/min per nanomole of P-450 in liver microsomes. ER *O*-deethylase activity (Table 3) was also fairly low, with turnover numbers in ventricle and atrium microsomes (0.4–0.6 nmole/min per nanomole of P-450) substantially lower than an average value of nearly 6 nmoles/min per nanomole of P-450 obtained with liver microsomes. Even in samples with P-450 content as high as 0.9 nmole/mg the rate of ER metabolism was very low. AP *N*-demethylase activity in ventricle was also low, and was quite comparable to the value seen previously in microsomes of whole heart (7). In animals treated with 3-MC under conditions known to induce hepatic P-450, the level of cardiac BP monooxygenase activity was somewhat higher than the mean value for the untreated fish we examined, but the difference was not statistically significant. BP monooxygenase in cardiac tissues was strongly inhibited by 7,8-BF (Fig. 4).

The values for BP monooxygenase presented in Table 3 represent data obtained by measuring fluorescent products and thus certainly underestimate the total turnover of BP, and in fact this activity in both ventricle and atrium was only about 20% of that obtained by measuring [^{3}H]BP metabolites in a standard radiometric assay. A graphic example of the pattern of BP metabolites formed *in vitro* by a sample of atrial microsomes is displayed in Fig. 5. Similar profiles were obtained with ventricle microsomes. The addition of 1,1,1-trichloropropene oxide to the reaction mixtures completely abolished the peaks eluting with 9,10-dihydrodiol and 7,8-dihydrodiol, with a concomitant increase in phenolic derivatives, implying that epoxide hydrolase is active in cardiac microsomes. The patterns of formation of BP metabolites *in vitro* presented in Table 4 indicate that the regiospecificity of such metabolism is quite similar in both ventricle and atrium microsomes. The 9,10- and 7,8-diol accounted for 50–70% of the total metabolites produced in each case. It is also notable that no detectable 4,5-dihydrodiol or 6,12-quinone was formed by either tissue. The evident lack of 4,5-dihydrodiol formation does not appear to be related to the procedure employed. Measured recovery (82%) of 4,5-dihydrodiol in procedural blanks was the same as that obtained with the other diols, and in our hands rat liver microsomes produced 4,5-dihydrodiol in amounts consistent with those previously reported (17).

DISCUSSION

It is clear from these results that microsomal fractions relatively uncontaminated by mitochondria from both muscular parts of untreated scup heart have rather high levels of P-450 with an optical absorption maximum shifted from that seen in other fish tissues, including the vascular tissue closely associated with the heart. Previous studies (7, 8) provided no definitive evidence that esti-

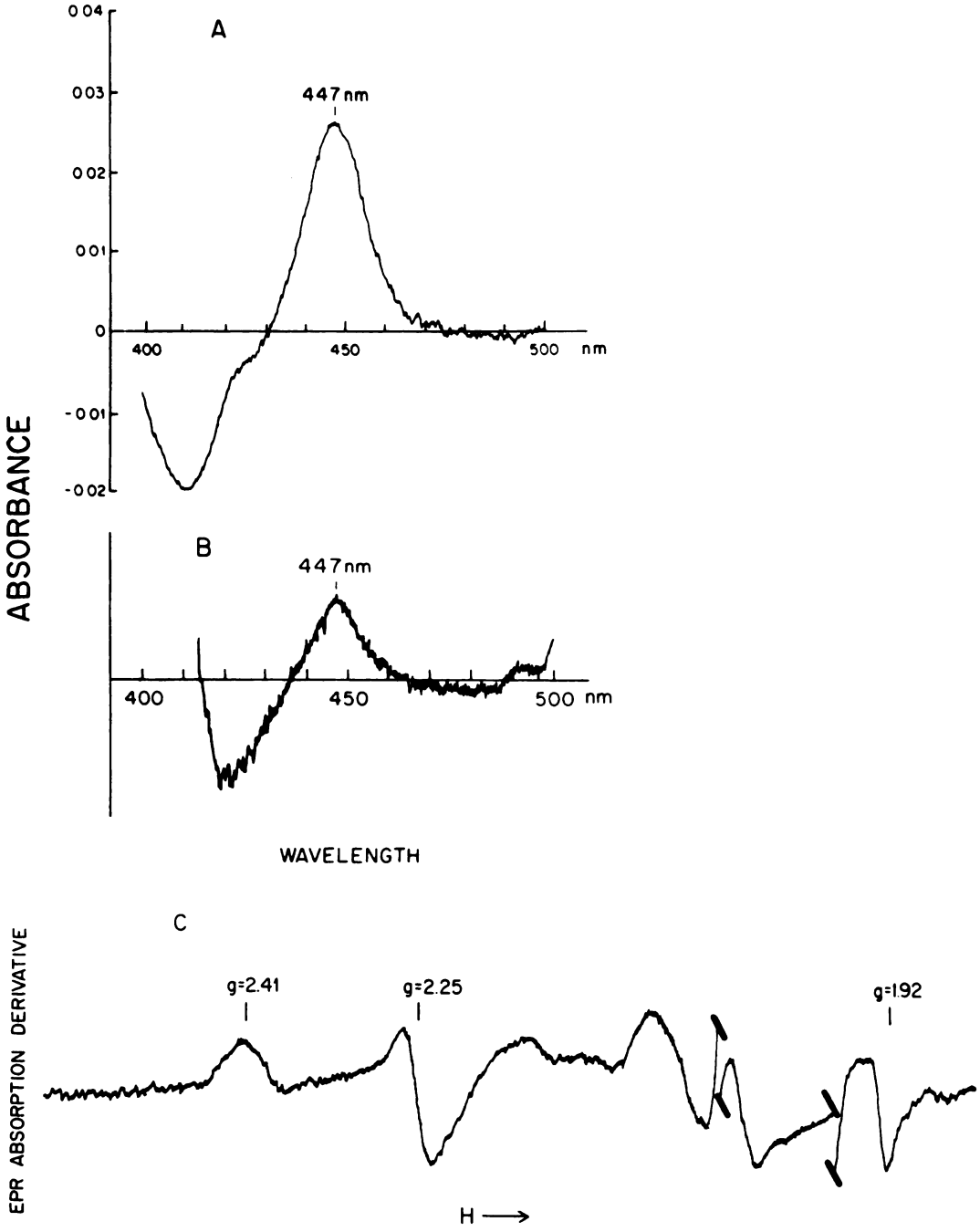

FIG. 3. *Difference spectra of atrial and ventricular microsomes of Stenotomus chrysops*

A. Dithionite difference spectrum of CO-treated atrial microsomes of *S. chrysops*. Protein in the assay was 1.18 mg/ml.

B. NADPH difference spectrum of CO-treated ventricular microsomes of *S. chrysops*. Protein in the assay was 0.91 mg/ml.

C. Low-temperature (14° K) EPR spectrum of *S. chrysops* ventricular microsomes. The cytochrome P-450 concentration was 3.2 μM. The conditions of EPR spectroscopy are given under Materials and Methods; the amplification where the line is broken in the region of $g = 2$ is 0.1 times the amplification of the main spectrum.

mates of either the amount of P-450 or its apparent optical properties were free of influence from other chromophores, even though in scup contamination by cytochrome oxidase was described as minimal (7). Using antiserum to hepatic P-450, Guengerich and Mason (8) demonstrated that P-450 was in fact present in their heart preparations, but the results of the analyses they employed were qualitative rather than quantitative. On the other hand, the analysis of EPR results presented here both establishes the presence of P-450 in cardiac microsomes and indicates that the levels estimated optically are substantially correct. Although rats and scup apparently can have high levels of P-450 in heart microsomes, the only other species in which P-450 has been reported in this tissue fraction, pig, had very low levels, about 0.013 nmole/mg of protein (18). It should be noted

that P-450 also occurs at low levels (approximately 0.004–7 nmoles/mg of microsomal protein) in rat aorta (19), a vascular structure like the vascular bulbus arteriosus of teleost fish.

In spite of the high levels of P-450 in scup atrium and ventricle, the turnover numbers for BP monooxygenase, ER *O*-deethylase, and AP *N*-demethylase in scup reported here, and BP monooxygenase and ethoxycoumarin *O*-deethylase in rat (8), were 10–30 times lower in heart microsomes than in those of liver. It is possible that reduction of P-450 by NADPH-cytochrome *c* (P-450) reductase could be a limiting factor in the heart monooxygenase activity seen here. The reduction of P-450 by NADPH added to CO-treated microsomes indicated that reducing equivalents were transferred via the flavoprotein. However, its role in determining monooxygenase rates can be further tested. It is more likely that the low monooxygenase activity simply reflects a low catalytic efficiency with the substrates chosen here. If so, then it is possible either that the catalytic activities observed are incidental functions of P-450 in heart microsomes or that the activities observed represent the primary function of a minor species of this P-450. Such a minor form might be constitutive or induced by some foreign compound. In studies with rats (8), 3-MC did elicit an induction of BP monooxygenase activity, but the increase was only 2- to 3-fold and it occurred with no change in the levels of cytochrome P-450, which would be consistent with induction of a minor form. Aromatic hydrocarbons can accumulate in fish heart tissue (20) and apparently elicit induction of BP monooxygenase activity there (21). The response to 3-MC in heart of scup, even though comparable to the response in rats (8), was limited. A limited response here could stem from prior induction of scup by environmental chemicals, a possibility suggested by studies with scup liver (11). The appearance of strong inhibition of BP monooxygenase by 7,8-BF and the presence of ER *O*-deethylase activity, both of which are generally associated with P-450s induced by 3-MC (22, 23), together with the low turnover numbers for BP and ER, would be consistent with the idea that there may have been some induction of a minor form of P-450 in scup heart in animals receiving no treatment. Alternatively, a longer treatment or a more potent inducer might produce a greater response in heart.

TABLE 2

EPR characteristics of Stenotomus chrysops cardiac microsomes

Sample microsomes[a]	Low-spin *g* values[b]		
Ventricle	2.41	2.25	1.92
Atrium	2.41	2.25	1.92
Liver	2.41	2.24	1.92

[a] N = 102 fish in one pool (ventricle), 105 fish in one pool (atrium), or 21 fish in one pool (liver).

[b] Samples contained P-450 at concentrations of 3.6 μM (atrium) or 3.2 μM (ventricle) determined by comparison of normalized peak heights of resonance at g_{max}, g_{mid}, and g_{min} for the heart samples with those obtained for a known amount of scup liver microsomal P-450.

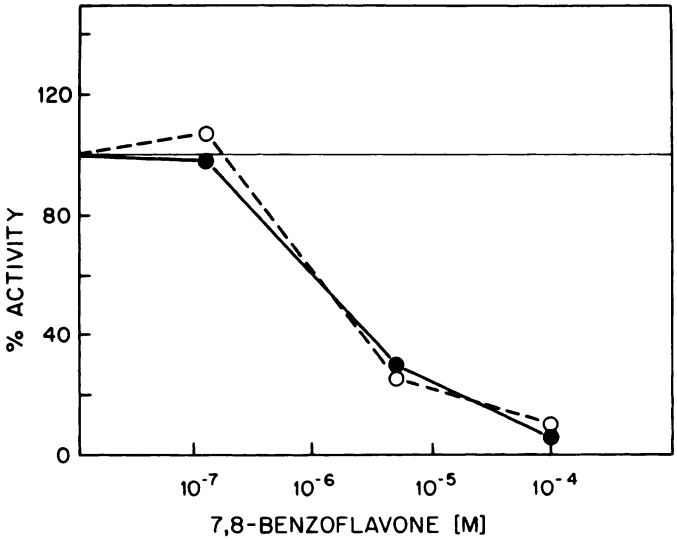

FIG. 4. *7,8-BF inhibition of Stenotomus chrysops ventricular BP monooxygenase activity*

Activities (100%) were 0.017 nmole of 3-OH-BP/min per milligram in the untreated samples (N = 15 fish in one pool) and 0.029 nmole/min per milligram in the treated samples (N = 12 fish in one pool). Data are means of replicate assays with a variability of ± 7%. ○, Untreated; ●, 3-MC treated.

TABLE 3

Cytochrome P-450 and microsomal enzyme activities in Stenotomus chrysops heart tissues

Component or activity	Ventricle	Atrium	Bulbus arteriosus	Liver[a]
Microsomal protein (mg/g tissue)	4.5 ± 2.4 (188,10)[b]	4.8 ± 2.1 (163,9)	3.5 ± 0.2 (26,2)	12.4 ± 1.2 (40,9)
Cytochrome P-450 (nmole/mg)	0.18 ± 0.06 (90,5)	0.25 ± 0.05 (63,3)	0.03 ± 0.01 (20,1)	0.61 ± 0.15 (38,9)
NADPH-cytochrome *c*[c] reductase (units/mg)	4.8 ± 1.2 (96,6)	7.1 ± 1.3 (96,6)	3.8 ± 0.3 (26,2)	107 ± 6 (30,5)
BP Monooxygenase[d] (units/mg)	0.017 ± 0.009 (71,4)	0.024 ± 0.015 (71,4)	0.005 ± 0.001 (26,2)	1.23 ± 0.08 (40,9)
ER *O*-deethylase[d] (units/mg)	0.07 ± 0.01 (102,1)	0.14 ± 0.01 (105,1)	—	3.7 ± 1.8 (17)[e]
AP *N*-demethylase[d] (units/mg)	0.33 ± 0.09 (33,1)	—	—	4.0 ± 0.5 (40,9)

[a] Liver data are from Stegeman *et al.* (7) except for ER *O*-deethylase activity, which was determined on individual fish that also served as a source of heart tissues.

[b] Numbers in parentheses refer to N fish from which tissue was pooled, in N pools. Values for three or more pools are expressed as means ± standard deviation. Those for one or two pools represent means ± range of replicates.

[c] Units are nanomoles of cytochrome *c* reduced per minute.

[d] Units are nanomoles of 3-OH-BP equivalents, resorufin or formaldehyde produced per minute.

[e] Value for liver ER *O*-deethylase activity is mean ± standard deviation of results with 17 fish assayed individually.

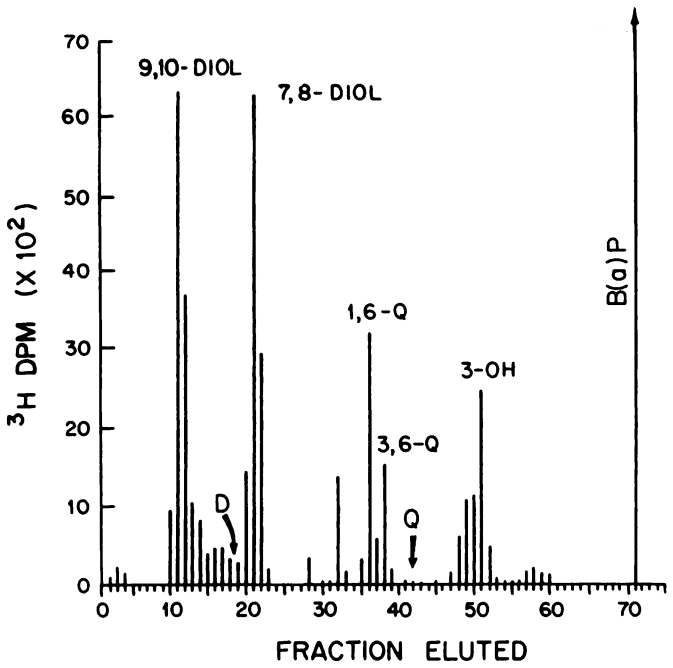

FIG. 5. *Elution profile of [^{3}H]BP metabolites formed by Stenotomus chrysops atrial microsomes*

Label appearing in analyses of complete reactions stopped at time zero was subtracted. The total label eluting with unmetabolized BP in this analysis was about 5.1 × 10^5 dpm. Less than 4% of the total substrate was metabolized within 20 min. Abbreviations indicated on the chromatogram are: *diol*, dihydrodihydroxy BP, *Q*, BP quinone; *OH*, hydroxy BP. *Arrows* indicate positions where authentic 4,5-diol (*D*) and 6,12-Q (*Q*) standards eluted.

TABLE 4

BP metabolite formation by cardiac microsomes of Stenotomus chrysops

Metabolite	% Total metabolites formed by	
	Ventricle[a]	Atrium[a]
9,10-Dihydrodiol	31 ± 9	39
7,8-Dihydrodiol	31 ± 17	27
1,6-Quinone	5 ± 4	12
3,6-Quinone	14 ± 8	5
Monohydroxy[b]	18 ± 15	16

[a] Values for ventricle represent the mean obtained with 133 fish in three pools, ± range. Values for atrium represent data obtained with one pool of 102 fish. The mean concentration of protein in reaction mixtures was 3.1 mg/ml (ventricle) or 3.6 mg/ml (atrium). Less than 5% of the total BP in the reaction mixture was metabolized with either ventricular or atrial microsomes. Activities (100%) were 11 pmoles/min per milligram and 18.0 pmoles/min per milligram, respectively.

[b] Compounds eluting 9-OH-BP or 3-OH-BP, which can include 1-OH-BP, 3-OH-BP, 7-OH-BP, and 9-OH-BP (19). A 9-OH-BP peak was not seen in some samples.

The regiospecificity of BP metabolism seen in both atrium and ventricle could offer further information about the P-450s in heart. A preference for metabolism on the benzo-ring is commonly associated with 3-MC-induced hepatic P-450s in mammals (16, 24). It is thus noteworthy that both atrial and ventricular microsomes formed high percentages of benzo-ring diols. However, hepatic P-450s in many untreated teleost fish also possess a capacity for efficient metabolism on the benzo-ring of BP (25), and this characteristic need not be linked to induced P-450 in fish.

Two additional points concerning the pattern of BP metabolism deserve attention. First, the lack of any detectable metabolism at position 4,5-(K-region) of BP in either atrium or ventricle of scup is also seen with liver microsomes of this species (25, 26), an intriguing coincidence. Second, a high degree of metabolism at the benzo ring is often associated with production of mutagenic diol-epoxides that could contribute to proliferative disease. Few cardiac diseases have been documented in fish, but we have observed two cases of pericarditis in scup, both of which exhibited extreme proliferation of the ventricular pericardium.[6] The etiology is not yet known, but further survey of scup for such disease may prove very interesting.

Considering the importance of known functions of P-450, the high levels of this hemoprotein in heart suggest that it may play an important role in cardiac physiology in fish, and probably in mammals. Although the levels of monooxygenase activity observed here were low, their presence clearly suggests a function for P-450 in xenobiotic metabolism in heart. However, the implication is also clear that there may be other function(s) of P-450 in heart. There could, for example, be functions related to steroid metabolism. The presence of androgen receptors in both atrial and ventricular myocardial cells of rats has been described recently (27), and ventricular tissue might carry out testosterone hydroxylation (28). Other molecules, e.g., catecholamines and cardioactive natural products such as digitoxin, can be hydroxylated by P-450 in liver (29, 30) and perhaps some related functions may occur in heart. However, whatever functions are served probably fluctuate, as levels of P-450 in scup heart were found to vary more than 10-fold. Further studies are in progress to determine the occurrence of cardiac P-450 in various species, the cell types in which it is located, and binding and metabolism of various potential substrates, which may provide clues to the function and regulation of this hemoprotein in heart.

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