Biochemical Pharmacology, Vol. 29, pp. 553–558.
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0006-2952/80/0215-0553 $02.00/0

SEX DIFFERENCES IN CYTOCHROME P-450 AND MIXED-FUNCTION OXYGENASE ACTIVITY IN GONADALLY MATURE TROUT

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(*Received* 16 *February* 1979; *accepted* 14 *August* 1979)

Abstract—Levels of microsomal cytochrome P-450 and aminopyrine demethylase activity in liver and of cytochrome P-450 in kidney of gonadally mature rainbow and brook trout were markedly greater in males than in females. Similar differences appeared in hepatic microsomal NADH- but not in NADPH-cytochrome *c* reductase activity or cytochrome b_5 content. When normalized to cytochrome P-450 content, benzo[*a*]pyrene hydroxylase activity in both liver and kidney was greater in females. In liver, there was a pronounced sex difference in the response of this activity to 7,8-benzoflavone, suggesting cytochromes P-450 of different catalytic function. Electron paramagnetic resonance spectra of hepatic microsomal cytochromes P-450 in mature brook trout were not demonstrably different between males and females, and crystal field parameters indicate that axial ligands to the heme are the same in these as in other cytochromes P-450. Mixed-function oxygenase activities in liver of gonadally immature brook trout differed from those in mature fish, and there was no sex difference. The appearance of seasonally dependent sex differences suggests that fish may provide interesting models for studying regulation of sex-specific forms of cytochromes P-450.

It is established that microsomal cytochrome P-450-dependent mixed-function oxygenase (MFO) are present in fish and that fish MFO, like mammalian MFO, are involved in the metabolism or biotransformation of endogenous compounds such as steroid hormones [1] and xenobiotics such as polynuclear aromatic hydrocarbons [2]. Variation in mammalian hepatic cytochrome P-450 is associated with differences in numerous biological and environmental factors [3]. MFO activity in fish also shows variation with species [4], strain [5], size [6] and chemical treatment or environment [6–9]. There are, however, no detailed reports treating sex-linked differences of MFO in fish,§ although some studies with trout [5, 11] have alluded to a predominant lack of such differences.

Most fish species are subject to annual reproductive cycles with periods of gamete development and spawning followed by periods of gonadal regression and inactivity. These annual cycles are generally accompanied by the appearance of extremes in metabolic and biochemical differences between males and females [12]. It is reasonable to expect that if sex differences in hepatic MFO do occur in fish, they would be most pronounced during periods of peak gonadal activity. In this report, we describe aspects of cytochrome P-450 and its catalytic functions, along with other characteristics of microsomal electron transport systems, in both gonadally mature (spawning) and gonadally immature (quiescent) brook trout and in mature rainbow trout.

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§ A preliminary version of this report has appeared [10].

MATERIALS AND METHODS

Chemicals. Benzo[*a*]pyrene (BP; Gold Label), aminopyrine (AP) and 7,8-benzoflavone (7,8-BF) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). NADP, NADPH, NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, horse heart cytochrome *c*, Tris and 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES) were obtained from the Sigma Chemical Co. (St. Louis, MO).

Animals. Brook trout (*Salvelinus fontinalis)* and rainbow trout (*Salmo gairdneri)* were obtained from the Sandwich Fish Hatchery, Massachusetts Division of Fisheries and Game. All fish had been spawned, hatched and reared at the Sandwich hatchery until sampling, and all members of given species were of the same genetic stock. After hatching, fish had been fed edible beef liver for 2 months until transfer to raceways. Fish in the raceways fed actively on Rangins Production Pellets (Zeigler Brothers, Gardners, PA) and Strike Pellets (Agway) twice daily at about 1.5 lb food/100 lb fish. The water supplying the hatchery is from artesian wells located within 100 yards of the raceways. The water temperature is a constant 10° at the source, but varies seasonally in the raceways between 5° and 11°.

Mature fish were pair-sampled in 1975, 1976 and 1978 between 25 October and 15 November, and quiescent brook trout were sampled in March and June of 1977. Brook trout were sampled in groups of small (100–260 g; 15-month to 2-year-old) and large (450–970 g; 4+years old) animals. Rainbow trout were all larger fish (400–770 g). The larger fish,

whether gravid or quiescent, had spawned at least twice at the hatchery. Small gravid fish were in their first or second season, and small quiescent fish were functional adults approaching their first season. Maturity of gametes in gravid fish was determined by stripping eggs and sperm prior to killing, and fertilizing eggs in the laboratory according to routine methods. Fertilization was judged as better than 98 per cent. Eggs were incubated in flowing well water at 11° until hatching, and the production of progeny was an absolute measure of gonadal maturity.

Fish were transported to the laboratory alive and maintained in their own water at 9° until use (within 24 hr). Fish were decapitated and liver and kidney were immediately excised and placed in ice-cold buffer. Tissues were homogenized in 5 vol. of 0.065 M phosphate buffer, pH 7.0, containing 3 μM $MgCl_2$ and 1.15% KCl, using a Potter–Elvehjem tissue grinder. Microsomal fractions, prepared as described previously [13], were resuspended in 0.1 M phosphate buffer, pH 7.3, to a concentration of 7–9 mg protein/ml.

Enzyme assays. NADPH-cytochrome *c* reductase was assayed at 25° by a modification of the method of Phillips and Langdon [14], with a reaction mixture containing 0.175 mM NADPH and 80 μM horse heart cytochrome *c* in 0.2 M potassium phosphate buffer, pH 7.7. Microsomal NADH-cytochrome *c* reductase was assayed using the conditions for NADPH-cytochrome *c* reductase, with 0.25 mM NADH replacing 0.175 mM NADPH. Reduction of cytochrome *c* was followed at 550 nm.

BP hydroxylase was assayed in a 1.0 ml reaction mixture containing 0.7 to 1.0 mg microsomal protein and an NADPH-generating system [6] in either 0.1 M phosphate or 0.1 M Tris–HCl with a final pH of 7.3. BP, with or without 7,8-BF, was added in 40 μl methanol to a final concentration of 60 μM, and incubated in a shaking water bath at 29° for 15 or 30 min. Products were assayed with a procedure similar to that of Nebert and Gelboin [15] with 3-OH-BP as a reference standard.

AP demethylase was assayed in a 1.5 ml reaction mixture containing an NADPH-generating system and microsomal protein as above in 0.166 M HEPES buffer, final pH 7.6. Reactions were initiated by adding 2× recrystallized AP in HEPES in a final concentration of 15 mM incubated as above. The formaldehyde generated was determined according to the method of Nash [16], as modified by Cochin and Axelrod [17]. Blanks for both BP hydroxylase and AP demethylase consisted of reactions without NADPH; the conditions for assay of both were determined to be optimal in this laboratory.

Microsomes were diluted to 0.6 to 1.0 mg protein/ml in 0.1 M phosphate buffer, pH 7.3, and cytochrome P-450 was analysed optically using a Cary 118-C recording spectrophotometer. CO-treated, $Na_2S_2O_4$-reduced microsomes occupied sample cuvettes; reference cuvettes held only CO-treated microsomes. Cytochrome b_5 was analysed in similar preparations, without CO, and sample cuvettes were reduced with 0.034 mg NADH/ml. Cytochrome content was estimated with the extinction coefficients of Omura and Sato [18].

Protein was determined according to the method of Lowry *et al.* [19], using bovine serum albumin as a standard. Analyses were performed in triplicate on individual animals or pooled samples, and data were analysed using standard *t*-tests performed on variance estimates [20].

Electron paramagnetic resonance (e.p.r.) analysis. Freshly prepared microsomal pellets containing 60–85 nmoles of cytochrome P-450 were frozen and stored in e.p.r. cavities in liquid nitrogen until analysis. The e.p.r. spectra were recorded at 1.6 K on a super-heterodyne spectrometer first described by Feher [21], using cavities first described by Berzofsky *et al.* [22]. Magnetic fields were recorded simultaneously with the e.p.r. spectrum using a digital Hall probe that had been calibrated against the proton resonance of water. Apparent *g* values were determined as described previously [23]. Using Griffith's crystal field analyses [24] adapted to the comparative study of low-spin ferric hemoproteins by Blumberg *et al.* [25, 26], the three *g* values were used to determine coefficients of two crystal field components, one of tetragonal symmetry, Δ, and the other of orthorhombic symmetry, V, relative to the spin orbit coupling constant, λ. The ratio of these coefficients (Δ and V, respectively) to spin orbit coupling energy (λ) determined two symmetry parameters, the tetragonality (Δ/λ) and the rhombicity (V/λ) of the heme.

RESULTS

The gonad–body weight ratios presented in Table 1 clearly describe the status of the gonadally mature and immature fish, and these ratios did not vary between large and small fish. Liver–body weight ratios were slightly higher in females than males, especially in gonadally immature fish. Liver hypertrophy in female fish is related to vitellogenesis [27], and the significant difference seen in immature fish may be due to the period of sampling, reflecting changes in metabolism necessarily preceding obvious gonadal development.

Reduced, CO-ligated cytochrome P-450 had a Soret peak at 450 nm in all female fish and in immature male fish, but in mature males the maximum occasionally appeared close to 449 nm. All male fish, however, had significantly greater content of cytochrome P-450 than did corresponding female fish (Table 1). When the data were normalized to body weight the sex differences remained highly significant in the mature animals but not in the gonadally immature brook trout. Neither cytochrome b_5 nor NADPH-cytochrome *c* reductase differed between the sexes in either species but NADH-cytochrome *c* reductase activity was significantly greater in males than in females of both.

Levels of hepatic AP demethylase in spawning males exceeded those in females by at least a factor of two (Table 1). However, a rough estimate of the turnover number (activity per nmole cytochrome P-450) was not different between male and female brook trout, but in rainbow trout there was a trend to a greater turnover number in moles. BP hydroxylase activity in mature fish was the same in both sexes, yet because of the differences in cytochrome content, the turnover number for BP hydroxylation in females was twice that in males. In gonadally immature fish,

Table 1. Hepatic microsomal electron transport components and mixed-function oxygenases in gonadally mature and immature trout

	Rainbow trout		Brook trout			
	Gonadally mature		Gonadally mature		Gonadally immature	
Characteristic	Male (9)*	Female (10)	Male (10)	Female (10)	Male (10)	Female (5)
Gonad wt/body wt(%)	2.0 ± 0.3†	15.5 ± 1.5	1.4 ± 0.4	17.0 ± 2.0	0.3 ± 0.1	0.7 ± 0.3
Liver wt/body wt‡ (%)	1.1 ± 0.2	1.2 ± 0.4	1.0 ± 0.2	1.3 ± 0.1	1.0 ± 0.2	1.6 ± 0.1§
Microsomal protein mg·g liver^{-1}	23.0 ± 3.0	23.9 ± 2.9	19.1 ± 3.0	18.1 ± 2.5	18.0 ± 2.3	19.7 ± 3.5
Cytochrome P-450 nanomoles·mg protein^{-1}	0.24 ± 0.02	0.13 ± 0.02‖	0.40 ± 0.03	0.19 ± 0.02¶	0.33 ± 0.04	0.20 ± 0.04‖
Cytochrome b_5 picomoles·mg protein^{-1}	36.0 ± 9.0	30.0 ± 6.0	46.0 ± 6.0	55.0 ± 8.0		
NADPH-cytochrome *c* reductase** units·mg protein^{-1}	22.1 ± 0.3	14.7 ± 8.3	36.8 ± 3.6	35.8 ± 4.7		
NADH-cytochrome *c* reductase** units·mg protein^{-1}	123.0 ± 8.0	63.0 ± 3.0‖	218.0 ± 9.0	166.0 ± 7.0‖		
AP demethylase††						
units·mg protein^{-1}	0.43 ± 0.08	0.16 ± 0.06‖	0.61 ± 0.10	0.28 ± 0.08‖	0.80 ± 0.31	0.59 ± 0.22
units·nmole P-450^{-1}	1.82 ± 0.38	1.17 ± 0.23‡‡	1.33 ± 0.20	1.65 ± 6.71	2.45 ± 0.90	2.92 ± 1.10
BP hydroxylase§§						
units·mg protein^{-1}	14.4 ± 4.7	15.3 ± 4.8	31.0 ± 8.2	30.1 ± 8.2	10.5 ± 4.4	6.0 ± 3.5
units·nmole P-450^{-1}	60.0 ± 21.0	130.0 ± 28.0‖	61.0 ± 23.0	136.0 ± 34.0‖	39.0 ± 13.0	28.0 ± 9.0

* Number of individuals. Reductase and cytochrome b_5 results were obtained with samples of three or four fish.
† ± S.D. of data obtained on individual fish.
‡ Calculated without gonad weight included.
§ Females significantly different from males at P ≤ 0.05.
‖ Females significantly different from males at P ≤ 0.01.
¶ Females significantly different from males at P ≤ 0.001.
** Units are nanomoles cytochrome *c* reduced/min.
†† Units are nanomoles HCHO produced/min.
‡‡ Females significantly different from males at P ≤ 0.02.
§§ Units are picomoles 3-OH-BP equivalents produced/min.

Table 2. Cytochrome P-450 and benzo[*a*]pyrene hydroxylase activity in kidney microsomes of gonadally mature trout*

Character	Rainbow trout Male (3)†	Rainbow trout Female (3)	Brook trout Male (4)	Brook trout Female (4)
Microsomal protein mg·g tissue^{-1}	3.8	4.0	5.1	6.2
Cytochrome P-450 picomoles·mg protein^{-1}	43.0 ± 3.0	24.0 ± 3.0	86.0 ± 8.0	59.0 ± 4.0
BP hydroxylase‡				
units·mg protein^{-1}	0.61 ± 0.3	0.80 ± 0.4	0.82 ± 0.3	1.03 ± 0.2
units·nmole P-450^{-1}	14.1	33.6	9.5	17.5

* Data represent mean of triplicate assays on samples of kidney pooled from three or four animals ± S.D. of the assay.
† Number of animals.
‡ Units are picomoles 3-OH-BP equivalents produced per minute.

there was no significant difference between males and females in either activity, whether normalized to microsomal protein or cytochrome P-450. The levels of hepatic microsomal electron transport components and MFO activities with both substrates, which were quite low in all fish, were higher in mature brook trout than in mature rainbow trout. Yet when normalized to cytochrome P-450, the MFO activities in mature fish were remarkably similar between the species. The levels of hepatic BP hydroxylase activity in quiescent fish were lower than those in the spawning fish.

Kidney tissue of mature fish was examined for sex differences in cytochrome P-450 content and BP hydroxylase activity (Table 2). Cytochrome P-450 in this tissue has a reduced, CO-bound Soret absorption maximum at 450 nm, and the levels of cytochrome P-450 appeared to be greater in males than in females. The levels of BP hydroxylase, based on microsomal protein, were extremely low in these samples, and a difference on this basis between the sexes could not be demonstrated. In both species there was a clear trend toward a greater turnover number in females, like that seen in liver.

The influence of 7,8-BF on hepatic BP hydroxylase *in vitro* [28] was assessed in mature males and females of both species (Table 3). In males, this activity was increasingly inhibited by increasing concentrations of 7,8-BF, up to about 50 per cent at 1×10^{-4} M. The latter concentration caused a similar inhibition in female brook trout, but there was no significant inhibition at lower concentrations of 7,8-BF. BP hydroxylase activity in rainbow trout females, on the other hand, was markedly stimulated by both 5×10^{-6} M and 1×10^{-4} M 7,8-BF.

These results suggest that the complement of

Table 3. Influence of 7,8-benzoflavone *in vitro* on hepatic microsomal benzo[*a*]pyrene hydroxylase in gonadally mature trout

7,8-Benzoflavone concentration (M)	Activity remaining (%)			
	Mature rainbow trout		Mature brook trout	
	Male (3)*	Female (3)	Male (10)	Female (10)
0	100†	100	100	100
1.25×10^{-7}	87.3 ± 3.0‡	89.1 ± 12.0	82.2 ± 12.0	92.7 ± 14.0
5.0×10^{-6}	70.1 ± 14.0	219.0 ± 64.0	73.4 ± 13.0	102.2 ± 14.0
1.0×10^{-4}	49.0 ± 11.0	134.0 ± 17.0	50.0 ± 21.0	40.0 ± 2.0

* Number of individuals.
† One hundred per cent in each case approximates the activity presented in Table 1.
‡ ± S.D. of triplicate assays on one (rainbow) or two (brook) pooled samples.

Table 4. Electron paramagnetic resonance characteristics of hepatic microsomal cyctochrome P-450 from gonadally mature brook trout*

Sample microsomes	N (pooled)	Absorption maximum (nm)†	Cytochrome P-450‡ mg microsomal protein	Low-spin g values
Male	8	450	0.381	2.409 2.244 1.911
Female	6	450	0.179	2.408 2.246 1.915

* Separate fish from those used for catalytic assays.
† Absorption maximum of reduced, CO-treated microsomes.
‡ Nanomoles of cytochrome P-450.

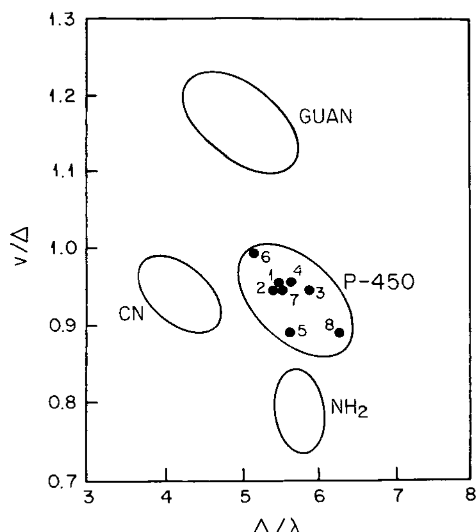

Fig. 1. Crystal field analyses of e.p.r. spectra of hepatic microsomal cytochromes P-450 from fish. The crystal field parameters, V/Δ and Δ/λ (rhombicity and tetragonality), are defined in Materials and Methods. Domains for cytochrome P-450 and other domains are as defined previously [26]. Numbers 1 and 2 are for brook trout males and females respectively. Numbers 3–8 are from data in Chevion *et al.* [9] for the marine fish *Stenotomus versicolor*. Key: (3) untreated; (4) corn oil-treated; (5) tricaine methanesulfonate-treated; (6) 3-methylcholanthrene-treated; (7) 5,6-benzoflavone-treated; and (8) 5,6-BF plus 3-methylcholanthrene-treated.

cytochromes P-450 was different in male and female trout liver. Electron paramagnetic resonance spectroscopy is very sensitive to changes in the spatial geometry and/or the electronic distribution around paramagnetic centers, like ferric iron in cytochrome P-450, and an alteration in the structure of the protein, even when originating far from the paramagnetic center, can be detected. Hepatic microsomes prepared from adult brook trout were analysed by e.p.r. spectroscopy, and the results are presented in Table 4. The principal features of e.p.r. spectra were essentially identical for males and females, with low-spin ($s = 1/2$) g values near 2.41, 2.245 and 1.91. There was no discernible high-spin ($s = 5/2$) cytochrome P-450 in either of these samples. The low-spin g values analysed in terms of crystal field parameters gave values for tetragonality (Δ/λ) near 5.4 and rhombicity (V/Δ) near 0.95 (Fig. 1).

DISCUSSION

It is quite clear from the present results that hepatic MFO in salmonid fish can differ between the sexes, and that the nature and extent of this difference are linked to gonadal maturity. The higher levels of hepatic cytochrome P-450 and AP demethylase activity in mature male, as compared to female, brook and rainbow trout are like the sex differences in these characters observed in post-pubertal rats of various strains [29–33]. In some strains [29], the activity of AP demethylase normalized to cytochrome P-450 content was also higher in males, a condition we found true as well for rainbow but not brook trout.

In rats, sex differences in hepatic BP hydroxylase activity are like those seen in AP demethylase; the activity in males is greater than in females whether normalized to protein or cytochrome P-450 [34, 35]. However, in mature trout, the sex difference in estimated turnover number for BP hydroxylation was not like AP demethylase. This discrepancy in sex-linked patterns of metabolism of different substrates in trout is nevertheless reminiscent of similar discrepancies in metabolism of Type I and other Type II substrates, such as aniline [29], in rats. Interestingly, the limited sex differences in rainbow trout MFO noted in passing by Pedersen *et al.* [5] concerned females in two of six strains that had greater hepatic aniline hydroxylase activity. However, the gonadal status of those animals was not indicated. The similarity between the patterns of sex differences in trout liver and kidney is also inconsistent with the situation in rats, which have a sex difference in renal BP hydroxylase activity opposite to that in the liver [35], and no sex difference in cytochrome P-450 content [33].

Much of the difference seen in hepatic demethylase activity between spawning male and female trout, in particular brook trout, might be explained by the quantitative differences in cytochrome P-450. However, the differences in BP hydroxylase turnover number and response to 7,8-BF argue persuasively that the cytochromes P-450 in males and females are not catalytically equivalent. NADPH-cytochrome *c* reductase activity did not differ between the sexes in trout, a condition also seen in rats [33], and consequently the ratios of reductase to cytochrome P-450 reflected the differences in turnover of BP. The reduction of cytochrome P-450 might thus be invoked as determining BP metabolism, but if there were not catalytic differences in the cytochromes we might expect AP demethylase to reflect this as well, which it clearly does not.

In a recent study [26], it was shown that all cytochromes P-450 exhibit very similar e.p.r. spectra leading to almost identical crystal field parameters indicative of the same structure; the heme is bound to histidine and mercaptide, presumably from cysteine. Such similarities notwithstanding, careful examination of e.p.r. spectra reveals some structure of the e.p.r. lines suggesting different heterogenous populations of cytochromes P-450 in mature male and female rats.* Unfortunately, analysis of the e.p.r. data here failed to reveal conclusively any differences between cytochromes P-450 in microsomes from male and female brook trout, the species with the most significant difference in cytochrome P-450 content. Our results do, however, demonstrate a similarity of hepatic microsomal low-spin ferric cytochrome P-450 from mature male and female trout with other cytochromes P-450 [26], including those from another teleost fish (Fig. 1) [9]. The crystal field parameters determined here suggest that mercaptide and imidazole are the axial ligands to the heme in fish cytochromes P-450 as well.

The sex differences in hepatic microsomal enzymes

* S. Fujita, M. Chevion and J. Peisach, personal communication.

in mammals have been attributed principally to the action of testosterone [36], apparently mediated by some pituitary factor [37, 38]. In rats, testosterone is stimulatory, resulting in greater MFO activity in males [36], and in some mice it is inhibitory, resulting in lower activity in males [39]. Testosterone is the most common C_{19} steroid in fish, and levels of circulating testosterone in some species have been found to fluctuate seasonally with gonadal cycles, peaking with spawning [40]. This can be regulated by the pituitary [41]. Thus, circulating steroids and possibly pituitary function may well be involved both in sex differences in MFO seen in fish, as they apparently are in mammals, and in the regulation of seasonal differences.

The results here also pertain to questions concerning the chemical environment and levels of hepatic MFO in fish. While several studies have reported very high levels of BP hydroxylase activity in trout species [5, 42], the activities seen here were quite low. The water in which fish in the present study lived until death was artesian, ostensibly pristine, and the low hydroxylase activity may reflect this. The results argue than that the high BP hydroxylase activities in some trout [42] and some marine fish species [13] might be environmentally induced. Nevertheless, the activity in these male trout was inhibited by 7,8-BF, although the inhibition was not as great as seen with BP hydroxylase from some other untreated trout [42, 43]. The 50 per cent inhibition seen with rainbow males was, however, like that seen with certain other untreated rainbow trout [44]. The appearance of 7,8-BF inhibition of BP hydroxylase activity in fish might be construed as indicative of partial induction by environmental chemicals. The same activity in mammals treated with aromatic hydrocarbon-like inducers is generally lower than in controls, and often inhibited, in the presence of 7,8-BF [28, 45]. It is clear, however, that not all fish show this characteristic, as the activity in rainbow females was stimulated by 7,8-BF. Thus, the origin of the strong inhibition in some fish, even those with low hydroxylase activity, remains uncertain.

Acknowledgements—This research was supported in part by NOAA Office of Sea Grant 04-6-158-44106 and by NSF grants OCE76-84415 and OCE77-24517. We thank Albert Sherman and Heidi Kaplan for fine technical assistance, and Lloyd Raymond, Massachusetts Division of Fisheries and Game, for providing the fish used here. Contribution No. 4304 from the Woods Hole Oceanographic Institution.

REFERENCES

1. H. Inano, K. Mori, B. Tamaoki and J-A. Gustafsson, *Gen. comp. Endoc.* **30**, 258 (1976).
2. L. Diamond and H. F. Clark, *J. Natn. Cancer. Inst.* **45**, 1005 (1970).
3. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
4. R. J. Pohl, J. R. Bend, A. M. Guarino and J. R. Fouts, *Drug Metab. Dispos.* **2**, 545 (1974).
5. M. G. Pedersen, W. K. Hershberger, P. K. Zachariah and M. R. Juchau, *J. Fish. Res. Bd Can.* **33**, 666 (1976).
6. J. J. Stegeman, *J. Fish. Res. Bd Can.* **35**, 668 (1978).
7. J. R. Bend, R. J. Pohl and J. R. Fouts, *Bull. Mt Desert Isl. biol. Lab.* **13**, 9 (1973).
8. J. F. Payne, *Science* **191**, 945 (1976).
9. M. Chevion, J. J. Stegeman, J. Peisach and W. E. Blumberg, *Life Sci.* **20**, 895 (1977).
10. J. J. Stegeman, *Fedn. Proc.* **36**, 941 (1977).
11. D. R. Buhler and M. E. Rasmusson, *Comp. Biochem. Physiol.* **25**, 223 (1968).
12. W. S. Hoar, *A. Rev. Physiol.* **27**, 51 (1965).
13. J. J. Stegeman and R. L. Binder, *Biochem. Pharmac.* **28**, 1686 (1979).
14. A. H. Phillips and R. G. Langdon, *J. biol. Chem.* **237**, 2650 (1962).
15. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **243**, 6242 (1968).
16. I. Nash, *Biochem. J.* **55**, 416 (1953).
17. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
18. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. R. R. Sokal and R. L. Rolf, *Biometry*, p. 166. W. H. Freeman & Co., San Francisco (1969).
21. G. Feher, *Bell Syst. tech. J.* **26**, 449 (1957).
22. J. A. Berzofsky, J. Peisach and W. E. Blumberg, *J. biol. Chem.* **246**, 3367 (1971).
23. J. Peisach, J. O. Stern and W. E. Blumberg, *Drug Metab. Dispos.* **1**, 45 (1973).
24. J. S. Griffith, *Proc. R. Soc.* **A235**, 23 (1956).
25. W. E. Blumberg and J. Peisach, in *Probes and Structure and Function of Macromolecules and Membranes* (Eds. B. Chance, T. Yonetani and A. S. Mildvan), Vol.2, pp. 215–229. Academic Press, New York (1971).
26. M. Chevion, J. Peisach and W. E. Blumberg, *J. biol. Chem.* **252**, 3637 (1977).
27. J. R. McBride and A. P. vanOverbeeke, *J. Fish. Res. Bd Can.* **28**, 485 (1971).
28. F. J. Weibel, J. C. Leutz, L. Diamond and H. V. Gelboin, *Archs. Biochem. Biophys.* **194**, 78 (1971).
29. J. B. Schenkman, I. Frey, H. Remmer and R. W. Estabrook, *Molec. Pharmac.* **35**, 516 (1967).
30. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 279 (1966).
31. R. Kato and K. Onoda, *Biochem. Pharmac.* **19**, 1649 (1970).
32. S. M. McLeod, K. W. Renton and N. R. Eade, *J. Pharmac. exp. Ther.* **183**, 489 (1972).
33. C. L. Litterst, E. G. Mimnaugh and T. E. Gram, *Biochem. Pharmac.* **26**, 749 (1977).
34. F. J. Weibel and H. V. Gelboin, *Biochem. Pharmac.* **24**, 1511 (1975).
35. H. L. Gurtoo and N. B. Parker, *Biochem. biophys. Res. Commun.* **72**, 216 (1976).
36. R. Kato, *Drug Metab. Rev.* **3**, 1 (1974).
37. R. E. Kramer, J. W. Greiner, W. J. Canaday and H. D. Colby, *Biochem. Pharmac.* **24**, 2097 (1975).
38. M. D. Burke, S. Orrenius and J-A. Gustafsson, *Biochem. Pharmac.* **27**, 1125 (1978).
39. J. Noordhoek, A. P. van den Berg, E. M. Savenije-Chapel and E. Koopman-Kool, *Xenobiotica* **8**, 515 (1978).
40. C. B. Schreck and M. L. Hopwood, *Trans. Am. Fish. Soc.* **93**, 375 (1974).
41. M. G. Drance, M. J. Hollenberg, M. Smith and V. Wylie, *Can. J. Zool.* **54**, 1285 (1976).
42. J. T. Ahokas, O. Pelkonen and N. T. Karki, *Biochem. biophys. Res. Commun.* **63**, 635 (1975).
43. J. T. Ahokas, O. Pelkonen and N. T. Karki, *Cancer Res.* **37**, 3737 (1977).
44. C. N. Statham, C. R. Elcombe, S. P. Szyjra and J. J. Lech, *Xenobiotica* **8**, 65 (1978).
45. S. S. Thorgeirsson, S. A. Atlas, A. R. Boobis and J. S. Felton, *Biochem. Pharmac.* **28**, 217 (1979).