Biochemical Pharmacology, Vol. 28, pp. 1681–1683.

0006-2952/79/0515–1681 $02.00/0

Equality of the rates of mixed function oxidation in livers of male and female chick embryos*

(*Received* 25 *May* 1978; *accepted* 8 *November* 1978)

Metabolism of drugs and foreign compounds is catalyzed principally by the hepatic microsomal mixed function oxidase system. The chicken embryo, which has proven to be useful in the study of heme and hemoprotein biosynthesis and metabolism, is potentially a very useful model for studying the regulation of mixed function oxidase activity as well [1–3]. The chick embryo contrasts with lower mammals in which hepatic drug-metabolizing enzyme activity is low or absent during fetal life and at birth and increases during early postnatal development. In the rat, for example, hepatic drug-metabolizing enzyme activity reaches adult levels at about 30 days postnatally in females and 60 days in males [4]. Aminopyrine *N*-demethylase activity undergoes a 500-fold increase during the first 5 weeks postnatally in the rat [5].

In the chick embryo, however, during the last 4 or 5 days prior to hatching (which occurs at 21 days), the hepatic drug-metabolizing enzyme activity is approximately equal to the activity in the adult chicken [6–9]. Since levels of drug metabolism in the adult chicken liver are 30–80 per cent of adult rat liver values [6, 8–10], the drug-metabolizing enzyme activity of chick embryo liver is substantial and is much greater than that of lower mammals at a comparable stage of development. Moreover, the mixed function oxidase system in chick embryo liver is highly sensitive to induction and inhibition by chemicals.

During studies of drug metabolism in the chick embryo, we have commonly observed about a 5-fold range in the rate at which livers from individual embryos of comparable ages carry out various mixed function oxidase reactions. We inquired whether sex differences might account for the individual differences in drug metabolism. Accordingly, we compared the rates of mixed function oxidase activity of livers from male and female embryos under V_{max} conditions in both the baseline (uninduced) and the induced state.

Chicken embryos, 18 days post-fertilization, of the White Leghorn strain were used. Sexual differentiation of the chick embryo is evident both morphologically and biochemically (by higher testosterone levels in the plasma of male embryos) from about 7.5 days [11, 12] post-fertilization. At 18 days, the characteristic differences in the size of the gonads of male and female chicks are grossly evident; therefore, sex assignment was made by inspection of the gonads after removing the livers. Livers were homogenized in 0.1 M potassium phosphate buffer, pH 7.4, and centrifuged at 9000 *g* for 15 min. The mixed function oxidase activities examined included aminopyrine demethylase, 7-ethoxycoumarin deethylase and aryl hydrocarbon hydroxylase (AHH). The reactions were measured as described [2, 13, 14] in the 9000 *g* supernatant fractions, under V_{max} conditions for the embryo, using substrate concentrations (μM) of 4000, 400 and 100 for aminopyrine, 7-ethoxycoumarin and benzo(a)pyrene respectively. Cytochrome P-450 was measured using the carbon monoxide difference spectrum of dithionite-treated microsomes [15] at a protein [16] concentration of 1.5 to 2 mg/ml of microsomal suspension, in a Cary model 15 spectrophotometer. An extinction coefficient of 91 mM^{-1} cm^{-1} for the difference in absorbance at 490 and 450 nm was used to calculate the concentration of P-450.

Though there was a wide range in the mixed function oxidase activity of individual livers, the mean values for aminopyrine demethylase, AHH and 7-ethoxycoumarin deethylase (Table 1) were not significantly different ($P > 0.05$) in male and female chick embryo livers, nor were there significant differences in the concentrations of cytochrome P-450. The carbon monoxide binding peak for cytochrome P-450 was at 450 nm in livers of both male and female chick embryos.

Allylisopropylacetamide (AIA) was used as an inducer of *N*-demethylase activity and cytochrome P-450, and β-naphthoflavone (β-NF) was used as an inducer of AHH, 7-ethoxycoumarin deethylase and P-448.

AIA (3 mg/egg), injected into the eggs 24 hr before dissection (Table 2), increased aminopyrine demethylase to 193 per cent of control values in livers from male embryos and to 163 per cent of control values in livers from females. While the mean increases in aminopyrine demethylase produced by AIA in livers of both male and female embryos were statistically significant ($P < 0.01$ and $P < 0.05$, respectively), the differences in mean control or induced aminopyrine demethylase levels of male and female livers were not statisti-

* This work was supported by grants from the New York Community Trust, the Revlon Foundation and the National Foundation.

Table 1. Mixed function oxidase activities of liver from male and female chick embryos*

Sex of embryo	Aminopyrine *N*-demethylase	AHH (nmoles/g liver/hr)	7-Ethoxycoumarin deethylase	Cytochrome P-450 (nmoles/mg microsomal protein)
Male	571 ± 67 (12) 223–1107	586 ± 77 (12) 227–1262	3411 ± 305 (12) 1896–5659	0.26 (2)
Female	760 ± 150 (12) 237–1990	461 ± 52 (12) 205–811	3333 ± 113 (12) 2656–3876	0.28 (2)

* Eighteen-day-old chick embryos were used; the number in parentheses represents the number of determinations. The means ± S.E. are given first; the range of values in the 9000 *g* supernatant fractions of individual livers are shown below the means. Aminopyrine *N*-demethylase [2], AHH [14] and 7-ethoxycoumarin deethylase [13] activities were each determined in triplicate. Activities are expressed as nmoles formaldehyde, phenols, or umbelliferone formed/g of liver/hr, respectively. P-450 [15] was determined in microsomes prepared from pooled 9000 *g* supernatant fractions from livers of male and female embryos. Two pools each containing the 9000 *g* supernatant fraction from six livers were prepared for males and two for females. The means for both pools are shown.

Table 2. Induction of mixed function oxidase activity by allylisopropylacetamide in livers of male and female chicken embryos*

Sex of embryo	Pretreatment	Aminopyrine *N*-demethylase (nmoles formaldehyde/g liver/hr)	Cytochrome P450 (nmoles/mg microsomal protein)
Males	Control	628 ± 112† (6)	0.24 (1)
	AIA	1215 ± 132 (12)	1.09 ± 0.14† (3)
Females	Control	738 ± 118 (6)	0.23 (1)
	AIA	1200 ± 150 (10)	0.90 ± 0.11 (3)

* Eighteen-day-old chick embryos were used; the number in parentheses represents the number of determinations. Three mg allylisopropylacetamide (AIA) in 0.2 ml water was injected into 17-day-old chick embryos 24 hr before dissection. Control embryos were injected with 0.2 ml water. Aminopyrine *N*-demethylase [2] was measured in 9000 *g* supernatant fractions of individual livers from control and AIA-injected embryos. Cytochrome P-450 determinations [15] for controls were made in microsomes prepared from pooled 9000 *g* supernatant fractions from the livers of the six male or six female embryos. For cytochrome P-450 in AIA-treated embryos, three pools of liver microsomes were prepared from males and three from females. Each pool was derived from the 9000 *g* supernatant fraction of livers from three or four embryos; P-450 was determined on each pool and the means are shown.

† Mean ± S.E.

cally significant ($P > 0.05$). AIA increased the cytochrome P-450 concentration 4.5-fold in livers of males and 3.9-fold in livers of females. Again the differences in the induced P-450 values of male and female livers were not statistically significant.

Similarly, β-NF (6.7 mg/egg), injected 24 hr before dissection (Table 3), increased AHH and 7-ethoxycoumarin deethylase to comparable extents in livers of male and female embryos (625 and 537 per cent compared to controls for AHH and 171 and 168 per cent for 7-ethoxycoumarin deethylase respectively). The induced AHH and 7-ethoxycoumarin deethylase activities were not significantly different in livers from male and female embryos. In chick embryo liver, as reported previously in rat liver [17], the carbon monoxide binding peak of the cytochrome induced by β-NF was decreased about 2 nm. The same spectral shift occurred in livers from both male and female embryos. The amount of increase in P-448 was also about the same in livers of male and female chick embryos (296 and 259 per cent of controls, respectively), and the mean induced levels of P-448 were not significantly different in livers of male and female embryos.

These studies show that the livers of chick embryos do not display significant sex differences either in the rate at which they carry out three model drug-metabolizing enzyme reactions or in the inducibility of those reactions. Sex differences in the rate of drug metabolism occur in rat liver [4, 5, 18]. Aminopyrine [4], 7-ethoxycoumarin [19] and benzo(a)-pyrene [20] are among the many substrates shown to be metabolized more rapidly by livers of male rats than by livers of female rats. Sex differences in metabolism of certain substrates by mouse liver have also been reported [21]. More often, however, sex differences have not been found in mouse liver nor in livers of other species, including guinea pigs and rabbits [22].

Sex differences in drug metabolism are not evident in the fetal rat but become apparent only after 3 or 4 weeks of age [4, 5, 23], when drug-metabolizing enzyme activity reaches levels about half of the adult values. Although the gestation periods of the rat and chick embryo are approximately the same (about 21 days), the hepatic drug-metabolizing enzyme activity of the fetal rat at 18 days gestation is low or undetectable, whereas the activity of the 18-day-old chick enbryo is comparable to the adult. Accordingly, if sex differences in drug metabolism occurred in the chicken, they might be expected to be evident in the 18-day-old embryo. However, we did not find any evidence for sex differences in the mixed function oxidase activity of chick embryo liver at that time. These findings, together with the finding of Drummond *et al.* [24] that chicks at 56 days postnatal age did not show sex differences in hepatic *N*-demethylase activity, suggest that sexual equality of drug-metabolizing enzyme activity is a characteristic of the chicken as a species, as well as of the particular stage of development studied here. Moreover, according to these findings individual differences in chick embryo liver drug metabolism cannot be attributed to differences of sex.

Acknowledgement—We thank Dr. William Greenlee and Dr.

Table 3. Induction of mixed function oxidase activity by β-naphthoflavone in livers of male and female chick embryos*

Sex of embryo	Pretreatment	AHH	7-Ethoxycoumarin deethylase	Cytochrome P-450
		(nmoles/g liver/hr)		(nmoles/mg microsomal protein)
Males	Control	1808 (2)	6598 (2)	0.27 (2)
	β-NF	11,300 ± 962† (10)	11,257 ± 629† (10)	0.80 ± 0.09† (4)
Females	Control	1808 (2)	6804 (2)	0.29 (2)
	β-NF	9723 ± 872 (10)	11,457 ± 359 (10)	0.75 ± 0.05 (5)

* Eighteen-day-old chick embryos were used; the number in parentheses represents the number of determinations. 6.7 mg β-Naphthoflavone (β-NF) in 0.1 ml dimethysulfoxide (DMSO) was injected into 17-day-old chick embryos 24 hr before dissection. Controls were injected with 0.1 ml DMSO. Each control determination of AHH [14] and 7-ethoxycoumarin deethylase [13] was made on the 9000 *g* supernatant fraction prepared from six livers pooled from control embryos, and P-450 [15] was measured in microsomes prepared from those supernatant fractions. For β NF-treated embryos, AHH and 7-ethoxycoumarin deethylase were measured in the 9000 *g* supernatant fractions from individual livers. Cytochrome P-450 in livers from β-NF-treated embryos was determined for males in four pools of microsomes and for females in five pools of microsomes, each prepared from the 9000 *g* supernatant fraction of livers from two to three embryos.

† Mean ± S.E.

Alan Poland for making their assay for 7-ethoxycoumarin dealkylation available to us prior to publication.

Departments of Pharmacology and Medicine,
Cornell University Medical College,
New York, NY 10021, U.S.A.

ARLEEN B. RIFKIND
MELODY TROEGER
TONIA PETSCHKE

REFERENCES

1. A. Poland and E. Glover, *Molec. Pharmac.* **9**, 736 (1973).
2. A. B. Rifkind, P. M. Gillette, C. S. Song and A. Kappas, *J. Pharmac. exp. Ther.* **185**, 214 (1973).
3. M. D. Maines and P. Sinclair, *J. biol. Chem.* **252**, 219 (1977).
4. P. Th. Henderson, *Biochem. Pharmac.* **20**, 1225 (1971).
5. S. M. MacLeod, K. W. Renton and N. R. Eade, *J. Pharmac. exp. Ther.* **183**, 489 (1972).
6. B. B. Brodie and R. P. Maickel, *Proc. First Int. Pharmac. Meeting* **6**, 299 (1961).
7. C. F. Strittmatter and F. T. Umberger, *Biochim. biophys. Acta* **180**, 18 (1969).
8. J. M. Machinist, E. W. Dehner and D. M. Ziegler, *Archs Biochem. Biophys.* **125**, 858 (1968).
9. G. Powis, A. H. Drummond, D. E. MacIntyre and W. R. Jondorf, *Xenobiotica* **6**, 69 (1976).
10. M. Hitchcock and S. D. Murphy, *Toxic. appl. Pharmac.* **19**, 37 (1971).
11. J. E. Woods, R. M. Simpson and P. L. Moore, *Gen. comp. Endocrin.* **27**, 543 (1975).
12. A. L. Romanoff, *The Avian Embryo*, pp. 816–35. Macmillan, New York (1960).
13. W. F. Greenlee and A. Poland, *J. Pharmac. exp. Ther.* **205**, 596 (1978).
14. D. W. Nebert and L. L. Bausserman, *J. biol. Chem.* **245**, 6373 (1970).
15. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. A. R. Boobis, D. W. Nebert and J. S. Felton, *Molec. Pharmac.* **13**, 259 (1977).
18. B. B. Brodie, *J. Pharm. Pharmac.* **8**, 1 (1956).
19. V. Ullrich, P. Weber and P. Wollenberg, *Biochem. biophys. Res. Commun.* **64**, 808 (1975).
20. R. S. Chhabra and J. R. Fouts, *Drug Metab. Dispos.* **2**, 375 (1974).
21. D. W. Nebert and H. V. Gelboin, *Archs. Biochem. Biophys.* **134**, 76 (1969).
22. R. Kato and K. Onoda, *Jap. J. Pharmac.* **16**, 217 (1966).
23. S. El Defrawy El Masry, G. M. Cohen and G. J. Mannering, *Drug Metab. Dispos.* **2**, 267 (1974).
24. A. H. Drummond, J. M. McCall and W. R. Jondorf, *Biochem. J.* **130**, 73P (1972).

Biochemical Pharmacology, Vol. 28, pp. 1683–1686.

0006-2952/79/0515-1683 $02.00/0

Antiviral activities of novel bridgehead C-nucleosides

(*Received* 9 *October* 1978; *accepted* 23 *November* 1978)

As a part of our program to develop chemotherapeutically useful analogs of the naturally occurring purines, with particular reference to the antiviral agents, the synthesis of several new C-nucleosides possessing bridgehead nitrogen has been accomplished. They include 3-β-D-ribofuranosyl-s-triazolo(4,3-a)pyrimidine (I) and 2-β-D-ribofuranosyl-s-triazolo(1,5-a)pyrimidine (II) basic systems which could be potential analogs of the antibiotics formycin A and B and their methylated derivatives (Fig. 1) [1, 2]. A major difference in structure between studied analogs and formycin is located at position 5 where bridgehead nitrogen is introduced; moreover, the NH function in pyrazole ring of analogs is lost, thus excluding the possibility of hydrogen bonding of the Watson–Crick type. Also, the pyrimidine part of the fused triazolo(4,3-a)pyrimidine in I does not possess the appropriate functions resembling formycin. However, it is known that isosteric 5,7-dimethylpyrazolo(1,5-a)pyrimidines act as potential inhibitors of 3′,5′-cyclic-AMP phosphodiesterase [3]. Therefore we suggest that the model compounds I and II might be useful for studying their substrate specificity in the antiviral test systems [4–7]. Furthermore, it has been proposed that the highly selective antiviral properties of some analogs, such as ara-adenine and ribavirin derivatives, can be attributed to their stability in the high anti-conformation [7]. In order to achieve such high anti-conformation with the compounds studied, a bulky group was introduced in I at peri position to the ribose moiety in fused aromatic heterocycle.

In the present report we described the antiviral effect of C-nucleosides with bridgehead nitrogen against some DNA and RNA containing viruses grown in mammalian cell cultures and their effects on the synthesis of cellular nucleic acids. This study was directed toward determination of the selective antiviral effects of analogs in relation to their conformation around the glycosidic bond.

The synthesis of C-nucleosides I and II whose structures are shown in Fig. 1, has been carried out as follows. Compound I (5,7-dimethyl-3-β-D-furanosyl-s-triazolo[4,3-a]pyrimidine) was prepared when 2-chloro-4,6-dimethyl pyrimidine was treated with 5-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl) (2H)-tetrazol in refluxing toluene for 30 hr and the isolated protected nucleoside was deblocked in NaOMe/MeOH solution. 5,7-Dimethyl-2-β-D-ribofuranosyl-s-triazolo[1,5-a]pyrimidine (II) was isolated when deblocking procedure was performed in methanolic ammonia. The ^{1}H n.m.r. and Nuclear Overhauser Enhancements (NOE) data for these analogs have been determined. 5,7-Dimethyl-2-β-D-ribofuranosyl-s-triazolo[1,5-a]pyrimidine: ^{1}H n.m.r. (deuterium oxide) δ-HMDS (capillary)—2.66 (s, 3, CH_3 [7]), 2.77 (s, 3, CH_3 [5]), 3.93 (m, 2, H2′H H5′), 4.20 (m, 1, H4′), 4.3–4.5 (m, 2, H2′H3′), 5.11 (d, 1, H1′), 7.15 (s, 1, H6) NOE $f_{H1'}$(CH_3 [5] saturated) = 0.

5,7-Dimethyl-3-β-D-ribofuranosyl-s-triazolo[4,3-a]-pyrimidine: ^{1}H n.m.r. (deuterium oxide) δ_{HMDS} (capillary)—2.53 (s, 3, CH_3 [7]), 2.88 (s, 3, CH_3 [5]), 3.68 (m, 2, H5′H5″), 4.16 (m, 1, H4′), 4.36 (dd, 1, H3′), 5.01 (dd, 1, H2′), 5.42 (d, 1, H1′), 6.92 (s, 1, H6) NOE $f_{H1'}$ (CH_3[5] saturated) = 0.17.

7-Methyl-2-β-D-ribofuranosyl-s-triazolo[1,5-a]pyrimidine: ^{1}H n.m.r. (deuterium oxide) δ_{TMS} (capillary)—8.39 (d, 1, H5, J_{56} = 7 Hz), 6.85 (d, 1, H6), 4.70 (d, 1, H1′, $J_{1'2'}$ = 5Hz), 3.73–4.08 (m, 3, H2′, H3′, H4′), 3.53 (m, 2, H5′H5″), 2.25 (s, 3, CH_3).

5-Methyl-3-β-D-ribofuranosyl-s-triazolo[4,3-a]pyrimidine: ^{1}H n.m.r. (deuterium oxide) δ_{TMS} (capillary)—2.64 (s, 3, CH_3), 3.36 (m, 2, H5′H5″), 3.84 (dd, 1, H4′), 4.04 (dd, 1, H3′), 4.66 (dd, 1, H2′), 5.07 (d, 1, H1′, $J_{1'2'}$ = 6 Hz), 6.58 (d, 1, H6), 8.06 (d, 1, H7, J_{67} = 4.5 Hz).

Additional ^{13}C n.m.r., NOE, and relaxation data for C-nucleosides synthesized will be published elsewhere.

The experiments reported in this paper were performed