in Q_{O_2} at 4 weeks. This observation, combined with the high Q_{O_2} in renal homogenates from 4-week-old rabbits, suggests that renal metabolic processes at this age are particularly susceptible to stimulation by substrates.

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REFERENCES

1. M. HORSTER and J. E. LEVY, *Am. J. Physiol.* **219,** 1061 (1970).
2. B. RENNICK, B. HAMILTON and R. EVANS, *Am. J. Physiol.* **201,** 743 (1961).
3. R. C. WILLIAMSON and E. P. HIATT, *Proc. Soc. exp. Biol. Med.* **66,** 554 (1947).
4. R. J. CROSS and J. V. TAGGART, *Am. J. Physiol.* **161,** 181 (1950).
5. J. B. HOOK, H. E. WILLIAMSON and G. H. HIRSCH, *Can. J. Physiol. Pharmac.* **48,** 169 (1970).
6. G. H. HIRSCH and J. B. HOOK, *J. Pharmac. exp. Ther.* **171,** 103 (1970).
7. J. K. KIM, G. H. HIRSCH and J. B. HOOK, *Pediat. Res.* **6,** 600 (1972).
8. W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER, in *Manometric Techniques*, p. 1. Burgess, Minneapolis (1957).
9. A. G. GORNALL, C. J. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177,** 751 (1968).
10. H. W. SMITH, N. FINKELSTEIN, L. ALIMINOSA, B. CRAWFORD and M. GRABER, *J. clin. Invest.* **24,** 388 (1945).
11. H. TOST, G. KOVER and E. SZOCS, *Acta physiol. hung.* **37,** 245 (1970).
12. J. B. HOOK, *Proc. Fifth Int. Congr. Nephrol.*, in press.

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Stoichiometry of drug metabolism in maturing male rats

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ANDROGENS are known to increase the rate of metabolism of type I drugs.[1–7] These reports indicate that androgens increase the binding of type I drugs to cytochrome P_{450} and thus increase the rate of reduction of cytochrome P_{450} and the rate of drug metabolism. However, recent studies by Hamrick *et al.*[8] on the relationship between NADPH oxidation and drug metabolism indicate that changes in the rate of metabolism of type I drugs are not always directly related to changes in the magnitude of type I spectral changes, the cytochrome P_{450} content, or the NADPH–cytochrome c reductase activity. These studies indicate that steroids such as testosterone, cortisone and spironolactone may regulate the rate of drug metabolism by altering the stoichiometric ratio between substrate-stimulated, carbon monoxide-inhibitable NADPH oxidation and drug metabolism.

The purpose of this study was to utilize the maturing male rat to study the effect of androgens on the stoichiometric ratio for ethylmorphine (EM) metabolism in the developing hepatic microsomal drug-metabolizing system, and to attempt to correlate the change in the stoichiometric ratio with changes in the binding and metabolism of EM. Since the testosterone concentration in the spermatic vein of male rats has been shown to increase from 0·42 μg/100 ml to 1·04 μg/100 ml between the age of 25 and 30 days and to increase from 0·96 μg/100 ml to 4·8 μg/100 ml between the age of 35 and 40 days,[9] the study was conducted in rats between the age of 3·0 and 6·5 weeks. Interestingly, the time of increase in testosterone concentration correlates well with previous observations that testicle size increases primarily between the age of 3 and 6 weeks,[10] that seminal vesicle size increases primarily between the age of 3 and 8 weeks,[10] that hexobarbital sleeping time decreases between the age of 4 and 5 weeks,[1] and that EM metabolism doubles between 3 and 5 weeks.[10] Thus, one might assume that the age of 3–5 weeks approximates puberty in the male rat.

For this investigation, a single group of male Sprague–Dawley rats weighing 40–50 g were obtained from Cherokee Lab Supply in Atlanta, Georgia, and they were utilized as they matured. As the animal

TABLE 1. ALTERATIONS OF THE HEPATIC MIXED FUNCTION OXIDASE SYSTEM IN MATURING MALE RATS*

Weight stage (g)	A_{max} (O.D.$_{423-470\,nm}$/ mg protein)	K_s (mM)	V_{max} (nmole/min/ mg protein)	K_m (mM)	NADPH oxidase (nmole/min/ mg.protein)	Stoichiometric relationship†
51–60	0·008 ± 0·0015	0·100 ± 0·0343	6·068 ± 0·653	1·645 ± 0·288	10·250 ± 0·891	0·6020 ± 0·081
81–88	0·027 ± 0·0049‡	0·397 ± 0·1224§	5·578 ± 0·292	1·813 ± 0·117	10·531 ± 2·123	0·5827 ± 0·099
105–115	0·022 ± 0·0019	0·240 ± 0·0308	6·690 ± 0·187§	1·560 ± 0·129	11·898 ± 1·016	0·5775 ± 0·061
125–135	0·017 ± 0·0015	0·086 ± 0·0114‡	11·260 ± 0·459‡	0·597 ± 0·031‡	9·247 ± 0·275§	1·2174 ± 0·030‡
160–170	0·010 ± 0·0018§	0·072 ± 0·0055	13·870 ± 0·359‡	0·398 ± 0·041‡	16·883 ± 0·993‡	0·8325 ± 0·063‡

* Values are the means ± standard error of four or six experiments. Each experiment was made with a microsomal preparation from the pooled livers of two or three animals.

$$\text{† Stoichiometric values} = \frac{V_{max}\ (N\text{-demethylation of ethylmorphine) in air}}{\text{Rate of NADPH oxidation in air with } V_{max} \text{ Concentrations of ethylmorphine, minus rate of NADPH oxidation in atmosphere of carbon monoxide without ethylmorphine.}}$$

‡ $P < 0{\cdot}01$ with respect to the immediate lower weight stage.
§ $P < 0{\cdot}05$ with respect to the immediate lower weight stage.

reached the age of 3·0 weeks (51–60 g), 3·5 weeks (81–88 g), 4·0 weeks (105–115 g), 5·5 weeks (125–135 g) and 6·5 weeks (160–170 g), groups of them were sacrificed by decapitation between 7:30 and 8:30 a.m. The rats were classified by weight as well as age to make the members of each group as similar as possible. After decapitation, the livers were removed and the hepatic microsomes were isolated as previously described.[11] Protein concentrations were determined by the biuret method as described by Gornall *et al.*[12] Spectral changes produced by binding of 0·055 mM to 1·833 mM EM to cytochrome P_{450} were measured in a Beckman model DK spectrophotometer. For binding studies, microsomes were washed by recentrifuging at 78,000 *g* for 1 hr and resuspending them in 0·05 M Tris buffer, pH 7·4, and 1·15% KCl. The K_s and A_{max} were determined by the method of Remmer *et al.*[13] EM metabolism was determined in incubation mixtures consisting of 4 mM $MgCl_2$, 12 mM glucose 6-phosphate, 1·00 enzyme unit of glucose 6-phosphate dehydrogenase/3 ml, 0·33 mM NADPH, 50 mM Tris buffer (pH 7·4), 5 mg microsomal protein/3 ml, and 0·31 mM EM, 0·62 mM EM, 1·25 mM EM, 2·50 mM EM or 5·0 mM EM. The mixtures were incubated in a Dubnoff metabolic shaker for 10 min at 37°C. The formaldehyde formed from the *N*-demethylation of EM was then estimated by the method of Nash.[14] The K_m and V_{max} were obtained by a least squares method as described by Davies *et al.*,[15] and NADPH oxidation was measured by the method of Gigon *et al.*[16] The stoichiometric relationship between substrate-stimulated, carbon monoxide-inhibitable NADPH oxidation and EM metabolism was determined by Index III of Stripp *et al.*[17] which is calculated as shown in the second footnote (†) to Table 1.

Data presented in Table 1 demonstrate that the initial and largest increase in the V_{max} occurs between the 105 and 115 g weight stage and the 125 and 135 g weight stage, or between 4·0 and 5·5 weeks of age, which approximates puberty in the male rat. So, changes in the various parameters of drug-metabolizing activity during this time period are of primary interest. Besides the increased V_{max} there is also a decrease in the K_m and in the K_s, no change in the A_{max}, a slight but significant decrease in NADPH oxidation, and an increase in the stoichiometric ratio. Interestingly, the magnitude of the increase in the V_{max} correlates well with the magnitude of the increase in the stoichiometric ratio, which indicates that the increase in drug metabolism is primarily due to an increased efficiency of the microsomal electron transport system. The fact that the apparent K_m and K_s decrease as the animals reach 125–135 g demonstrates that the increase in efficiency of the microsomal drug-metabolizing system at puberty correlates with an increased affinity of the cytochrome P_{450} for EM. Yet, the data demonstrate that the amount of EM bound to the hepatic microsomes does not increase as the rats reach 125–135 g.

These studies indicate that the increased metabolism of EM seen at puberty is related to an increase in the efficiency of the hepatic microsomal system, and that the increased efficiency of the system correlates well with an increased affinity of the microsomal system for EM. So, one might postulate that in the prepubertal rat, abortive substrate–cytochrome P_{450} complexes are formed, and that androgens, by increasing the affinity of cytochrome P_{450} for its substrate, decrease the number of abortive substrate–cytochrome P_{450} complexes to increase the rate of drug metabolism. The decrease in the number of abortive substrate–cytochrome P_{450} complexes is reflected in the increase in the stoichiometric ratio.

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REFERENCES

1. G. P. QUINN, J. AXELROD and B. B. BRODIE, *Biochem. Pharmac.* **1,** 152 (1958).
2. P. L. GIGON, T. E. GRAM and J. R. GILLETTE, *Biochem. biophys. Res. Commun.* **31,** 558 (1968).
3. D. D. LOTLIKAR, *Biochem. J.* **120,** 409 (1970).
4. S. D. MURPHY and K. P. DUBOIS, *J. Pharmac. exp. Ther.* **124,** 194 (1958).
5. R. KATO, E. CHIESARA and G. FRONTINO, *Biochem. Pharmac.* **11,** 221 (1962).
6. R. KATO, E. CHIESARA and P. VASSANELLI, *Jap. J. Pharmac.* **12,** 26 (1962).
7. R. KATO and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **150,** 279 (1965).
8. M. E. HAMRICK, N. ZAMPAGLIONE, B. STRIPP and J. R. GILLETTE, *Biochem. Pharmac.* **22,** 293 (1973).
9. D. W. KNORR, T. VANHA-PERTTULA and M. B. LIPSETT, *Endocrinology* **86,** 1298 (1970).
10. T. E. GRAM, A. M. GUARINO, D. H. SCHROEDER and J. R. GILLETTE, *Biochem. J.* **113,** 681 (1969).
11. B. STRIPP, M. HAMRICK, N. ZAMPAGLIONE and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **176,** 766 (1971).
12. A. G. GORNALL, C. H. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177,** 751 (1949).
13. H. REMMER, J. B. SCHENKMAN, R. W. ESTABROOK, H. A. SESAME, J. R. GILLETTE, S. NARASIMIHULU, D. Y. COOPER and O. ROSENTHAL, *Molec. Pharmac.* **2,** 187 (1966).
14. T. NASH, *Biochem. J.* **55,** 416 (1953).
15. D. S. DAVIES, P. L. GIGON and J. R. GILLETTE, *Biochem. Pharmac.* **17,** 1865 (1968).
16. P. L. GIGON, T. E. GRAM and J. R. GILLETTE, *Molec. Pharmac.* **5,** 109 (1969).
17. B. STRIPP, N. ZAMPAGLIONE, M. HAMRICK and J. R. GILLETTE, *Molec Pharmac.* **8,** 189 (1972).