Preparation of (±)-Laudanine (IV) from I—Sodium borohydride (0.1 g.) was added in portions to a solution of I (0.1 g.) in ethanol and the mixture was stirred at room temperature for 3 hr. Methanol was added and after 30 min. the reaction mixture was evaporated to dryness. The residue was dissolved in water and extracted with ether. The ether extracts were dried and evaporated to yield a residue which crystallized from methanol as white prisms of IV, m.p. 166–168°; $\lambda_{max.}^{EtOH}$ 230 and 283 nm. (log ε 4.08 and 3.78); positive $FeCl_3$ test, violet Burger test; mass spectrum: *m/e* 343 (1), 342 (5), 327 (2), 206 (100), 204 (8), 192 (40), 190 (80), 174 (8), 162 (36), and 137 (19); NMR ($CDCl_3$): δ2.52 s (3H, N—CH_3), 3.58 s (3H, C-7, OCH_3), 3.85 s (6H, C-6, C-4′, OCH_3), 6.08 s (1H, C-8*H*), 6.58 s (1H, C-5*H*), 6.52 and 6.73 *AB* quartet (2H, *J* = 8 Hz., C-2′, C-3′*H*), and 6.75 broad s (1H, C-5′*H*); (±)-laudanine lit. (1) m.p. 166–167°, Burger test: violet color (1).

Interconversion of Palaudine and I—A solution of V (3 mg.) and methyl iodide (0.1 ml.) in ethanol (2 ml.) was allowed to stand for 18 hr. It was evaporated and passed through an ion-exchange column (Cl^- form) using ethanol–acetone. The residue was crystallized from ethanol as needles (4 mg.), m.p. 231–233°. Palaudine methochloride and I were indistinguishable in terms of the R_f values and UV spectra, and no mixed melting-point depression was observed.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 10, 1971, from the *Department of Chemistry, Pennsylvania State University, University Park, PA 16802*

Accepted for publication October 27, 1971.

The authors are grateful to the National Institutes of Health for Grant CA-11450, and to Professor E. Brochmann-Hanssen for a sample of palaudine.

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Metabolism of Aniline and Hexobarbital by Liver Homogenates of Spironolactone-Pretreated Male Rats

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Abstract ☐ Spironolactone pretreatment increases aniline hydroxylation and decreases hexobarbital metabolism, with respect to the saline-treated controls, by liver homogenates of male rats. The effect of spironolactone pretreatment on the metabolism of a substrate is related to the sex dependence or independence of that substrate's metabolism. No spironolactone-induced changes in microsomal protein, cytochrome P-450 content, or carbon monoxide-induced P-450 difference spectra were observed.

Keyphrases ☐ Aniline—metabolism by liver homogenate of spironolactone-pretreated male rats ☐ Hexobarbital—metabolism by liver homogenate of spironolactone-pretreated rats ☐ Spironolactone pretreatment—effects on aniline and hexobarbital metabolism, liver homogenates, male rats

The effect of spironolactone on the NADPH-dependent microsomal hydroxylation pathway of drug metabolism has been the subject of several recent investigations. Solymoss *et al.* (1) and, more recently, Stripp *et al.* (2) showed that spironolactone enhances hexobarbital degradation in female rats. However, the latter investigators also showed that spironolactone retards hexobarbital degradation in male rats (2). These results suggest that the effect of spironolactone on drug metabolism is a function of sex.

The metabolism of hexobarbital in the rat is sex dependent (3), but the metabolism of aniline in this animal is independent of sex (4). Therefore, the authors investigated the *in vitro* metabolism of these substrates in rat liver homogenates from male animals pretreated with spironolactone, hoping to obtain insight into the nature of this drug's effect upon the metabolism of other drugs.

MATERIALS AND METHODS

Animals—Male weanling rats[1], maintained on Purina Laboratory Chow and tap water *ad libitum*, were used experimentally upon attaining a weight of 125–135 g. One group of seven experimental animals was injected intraperitoneally with 100 mg./kg. spironolactone[2] in saline twice daily for 4 days, according to the method of Stripp *et al.* (2). Another group of seven control animals received isotonic saline injections. Animals were fasted overnight prior to use and were sacrificed 16 hr. after the final injection.

Liver Homogenates—Animals were sacrificed, and 10% liver homogenates were prepared in a 0.25 *M* sucrose solution containing 0.05 *M* tromethamine chloride (pH 7.4), 0.005 *M* $MgCl_2$, and 0.010 *M* NaCl as previously described (5).

Aniline Incubations—Aniline (200 μmoles in 0.10 ml. 95% ethanol) was added to a 125-ml. conical flask containing 1.25 ml. of the 0.25 *M* sucrose solution described, which also contained 4 μmoles of $NADP^+$, 14 mg. of glucose 6-phosphate, 0.015 *M* nicotinamide, and 0.1% bovine serum albumin. Ten milliliters of 10% liver homogenate was then added, followed by 5 units (0.5 ml.) of glucose 6-phosphate dehydrogenase in the 0.25 *M* sucrose solution. This reaction mixture was then incubated and assayed for the *p*-aminophenol formed after 20 min. by the method of Kato and Gillette (4). Results were expressed in nmoles of *p*-aminophenol that formed per minute per milligram microsomal protein ±*SE* from six determinations.

Hexobarbital Metabolism—Hexobarbital (1 μmole in 0.10 ml. 95% ethanol) was added to 125-ml. conical flasks containing the incubation medium and the NADPH-generating system already described. The homogenate and the enzyme were added as described for the aniline experiments, and the reaction mixtures were incubated and assayed for hexobarbital disappearance after 1 hr. by the method of Cooper and Brodie (6). Results were expressed in nmoles of hexobarbital that disappeared per minute per milligram microsomal protein.

[1] Obtained from Sasco, Inc., Omaha, Neb.
[2] Obtained from Calbiochem.

Table I—Effect of Spironolactone Pretreatment of Male Rats on Microsomal Protein, Cytochrome P-450 Content, and Aniline and Hexobarbital Metabolism

Pretreatment	Microsomal Protein, mg./g. Liver	Cytochrome P-450, nmoles/mg. Microsomal Protein	Aniline Metabolism, nmoles/min./mg. Microsomal Protein	Hexobarbital Metabolism, nmoles/min./mg. Microsomal Protein
Spironolactone	26.21 ± 0.81[a]	1.084 ± 0.022[a]	43.0 ± 0.17[b]	0.309 ± 0.031[c]
Saline	26.38 ± 0.49	1.081 ± 0.022	24.4 ± 0.17	0.512 ± 0.025

[a] Difference not significant. Values are mean of five determinations ± *SE*. [b] 76.3% increase, $p < 0.01$. Values are mean of six determinations ± *SE*. [c] 39.8% decrease, $p < 0.05$. Values are mean of six determinations ± *SE*.

Determination of Microsomal Protein and Cytochrome P-450—The microsomal fraction was isolated from portions of the 10% liver homogenates by the method of Stohs *et al.* (5). Microsomes equivalent to 500 mg. of liver were solubilized with 5% sodium deoxycholate and assayed by the Lowry *et al.* method (7) for protein content, employing crystalline bovine serum albumin as the standard. Microsomes equivalent to 6 g. of liver were resuspended in 20 ml. of 1.15% KCl and assayed for cytochrome P-450 content by the method of Omura and Sato (8). The carbon monoxide cytochrome P-450 difference spectrum was scanned, using suspensions of microsomes from both saline- and spironolactone-pretreated animals[3]. Student's *t* test was employed to compare all results from saline- and spironolactone-pretreated animals.

RESULTS AND DISCUSSION

The results of this study support those previously reported (2) in that spironolactone pretreatment does not significantly change microsomal protein or cytochrome P-450 content, and it retards the *in vitro* metabolism of hexobarbital by male rat liver homogenates (Table I). However, a significant increase in aniline hydroxylation following spironolactone pretreatment was observed. Because hexobarbital and aniline induce Types I and II cytochrome P-450 spectral changes, respectively (9), one might conclude that the effect of spironolactone upon the metabolism of a particular substrate depends upon the type of cytochrome P-450 spectral change effected by that substrate. However, Stripp *et al.* (2) observed that spironolactone pretreatment increases *in vitro* hexobarbital metabolism in castrated male rats and in female rats and decreases *in vitro* hexobarbital metabolism in male rats and methyltestosterone-pretreated female rats. These latter observations indicate that the androgen dependence of a substrate's metabolism, not the type of cytochrome P-450 spectral change it induces, determines whether spironolactone pretreatment will enhance or retard its metabolism by the liver.

Metabolic results, qualitatively similar to the results reported here, were obtained by Kato *et al.* (10), using 3-methylcholanthrene-pretreated male rats. These investigators further observed that 3-methylcholanthrene did not increase microsomal protein; however, it did significantly increase cytochrome P-450 content (10). Alvares *et al.* (11) observed that 3-methylcholanthrene pretreatment caused a shift in the absorption maximum of the cytochrome P-450 carbon monoxide difference spectrum, and they (11, 12) presented evidence that this shift reflects the synthesis of a new hemoprotein. These authors suggested that the 3-methylcholanthrene-induced metabolism of some substrates may be related to their binding with this new hemoprotein in the NADPH-dependent microsomal hydroxylation pathway. In contrast, the authors did not observe a shift in the absorption maximum of the cytochrome P-450 carbon monoxide difference spectrum, indicating that spironolactone does not induce the synthesis of a new hemoprotein with spectral properties slightly different from those of cytochrome P-450. The two spectra were superimposed.

In conclusion, spironolactone pretreatment increases the *in vitro* metabolism of aniline, a substrate which is metabolically sex independent, by male rat liver homogenates. These results also support earlier findings that the metabolism of hexobarbital, which is sex dependent, is decreased by spironolactone pretreatment in the male rat. The antiandrogenic properties of spironolactone (13) may account for the retardation of hexobarbital metabolism in the male rat, but the mechanism by which spironolactone enhances aniline metabolism remains obscure. Because no spironolactone-induced change was observed in either microsomal protein content, microsomal cytochrome P-450 content, or carbon monoxide-induced cytochrome P-450 difference spectra, none of these parameters can be implicated in the spironolactone-induced enhancement of the *in vitro* metabolism of aniline in the male rat.

[3] A Beckman DBG spectrophotometer equipped with a Sargent recorder was used for the spectrophotometric assays involved in these experiments.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 13, 1971, from the *Department of Pharmacognosy, College of Pharmacy, University of Nebraska, Lincoln, NB 68508*

Accepted for publication November 19, 1971.

Supported in part by a National Institutes of Health Predoctoral Fellowship, 5 F01 GM47428, from the National Institute of General Medical Sciences.

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