Biochemical Pharmacology, Vol. 22, pp. 293–310. Pergamon Press, 1973. Printed in Great Britain.

INVESTIGATION OF THE EFFECTS OF METHYLTESTOSTERONE, CORTISONE AND SPIRONOLACTONE ON THE HEPATIC MICROSOMAL MIXED FUNCTION OXIDASE SYSTEM IN MALE AND FEMALE RATS

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(*Received* 6 *February* 1972; *accepted* 30 *June* 1972)

Abstract—Acute (4 days) and chronic (4 weeks) administration of methyltestosterone, cortisone and spironolactone changes drug metabolism in hepatic microsomes of male and female rats by altering the degree of binding of drugs to the cytochrome P-450, the activity of NADPH-cytochrome *c* reductase, and the rate of reduction of the cytochrome P-450–drug complex. These steroids also alter the ratio of stoichiometric relationship between drug metabolism and CO-inhibitable NADPH-oxidation. This ratio is sex dependent, being approximately 1·0 in males and 0·2 in females. The effect of the three steroids on the ratio is also sex dependent. The steroids tend to increase the ratio in females and decrease it in males.

SEVERAL studies on the regulation of hepatic drug-metabolizing activity by various steroids have been carried out after replacement therapy in animals that had been adrenalectomized,[1–5] gonadectomized[6–12] or both.[4] These and other studies involving administration of antagonistic gonadal hormones[8,13] revealed that androgens play an active role in the control of microsomal enzyme activity, whereas estrogen seems to have little or no effect in this respect. Furthermore, corticosteroids have been shown to increase drug-metabolizing activity to a greater extent in adrenalectomized male rats[1,4,14] than in adrenalectomized female rats.[9,15]

It has also been shown that a single intravenous dose of adrenocorticotropic hormone (ACTH) or corticosterone[16] decreases the hexobarbital sleeping time in male rats and that exposure to stress increased the *N*-demethylation of ethylmorphine.[17] On the other hand, a 4-day treatment of male rats with corticosterone impairs the *N*-demethylation of aminopyrine.[15] Recently, spironolactone, a steroid presumably without hormonal effects, has been shown to increase drug-metabolizing activity in female rats,[18] whereas the activities of these enzymes in male rats were increased to a much smaller extent or even decreased.[19] Recent studies have indicated that androgens in castrated male rats increase the binding capacity of drugs to liver microsomal

* Supported by special fellowship 5-FO-3-GM-37616-02, National Institute of General Medical Sciences.

† Supported by special fellowship 5-FO-3-GM-40512-02, National Institute of General Medical Sciences.

cytochrome P-450[7,8] and that corticosteroids in adrenalectomized male rats increase the activity of NADPH-cytochrome *c* reductase.[1] Similar studies on the effect of androgens or corticosteroids in intact male rats have not been carried out. It therefore seemed of interest to study the effect of acute or chronic administrations of methyltestosterone, cortisone or spironolactone on the oxidation system in the liver from male and female rats.

METHODS

Groups of male and female Sprague–Dawley rats were acutely or chronically pretreated with equal doses of methyltestosterone, cortisone acetate or spironolactone. Cortisone acetate, rather than corticosterone, which is predominant in the rat, was used because the former had a longer half-life. For acute pretreatments, 100 mg/kg of the drugs, suspended in saline by means of a few drops of Tween 80, was injected intraperitoneally twice a day for 4 days. This dosage was selected because earlier experiments had shown it to be optimal with respect to spironolactone. Males weighed 160–180 g and females weighed 120–160 g. For chronic pretreatments, 20 mg/kg of the drugs in corn oil was injected subcutaneously each day for 4 weeks. This dosage had been shown[20] to be optimal when female rats were treated for 4 weeks with methyltestosterone or when adrenalectomized male rats were treated for 4 days with cortisone acetate.[4] Males and females initially weighed 50–80 g, but at the termination of the experiment, with methyltestosterone or spironolactone, males weighed 160–180 g and females weighed 140–160 g. The animals, pretreated with cortisone acetate, however, weighed approximately 120 g at the end of the experiment. This decrease in weight was statistically significant, but the animals appeared otherwise normal. All animals were kept on a Purina diet with free access to water.

The animals were decapitated between 7 and 8 a.m. The livers were removed and immediately homogenized with 2 vol. of cold Tris–HCl–KCl solution (0·02 M Tris buffer, pH 7·4 and 1·15% KCl) in a Teflon–glass homogenizer. The homogenates were centrifuged for 20 min at 9000 *g* in a Sorvall refrigerated centrifuge and the supernatants then centrifuged for an hr at 78,000 *g* in a Beckman model L250 ultracentrifuge. The microsomal pellets were resuspended in the Tris–HCl–KCl solution to volumes corresponding to approximately twice the original liver weight.

The amounts of cytochrome P-450 were determined as described by Omura and Sato.[21] The spectral changes caused by substrates of the microsomal enzymes were measured in an Aminco-Chance dual-wavelength spectrophotometer, and the K_s and the A_{max} were calculated as described by Remmer *et al.*[22] Protein was determined according to the biuret method of Gornall *et al.*[23] The NADPH-cytochrome *c* reductase was measured according to Phillips and Langdon.[24] The NADPH cytochrome P-450 reductase and the NADPH oxidase were assayed by the method of Gigon *et al.*[11] A model 2000 Gilford spectrophotometer was used for these determinations.

Drug metabolism was determined in incubation mixtures consisting of 5 mM $MgCl_2$, 12 mM glucose 6-phosphate, 1·00 unit of glucose 6-phosphate dehydrogenase/3 ml, 0·33 mM nicotinamide adenine dinucleotide phosphate (NADP), 50 mM Tris buffer, pH 7·4, and various amounts of substrate and microsomes. The amounts of microsomal protein used per 3 ml were 5 mg for the studies with ethylmorphine and 6–10 mg for those with hexobarbital. Ethylmorphine concentrations used were 0·31,

0·62, 1·25, 2·50 and 5·0 mM. Hexobarbital concentrations were 0·15, 0·20, 0·25, 0·30, 0·40 and 0·60 mM. The substrates were incubated in a Dubnoff metabolic incubator at 37° for 10 min in air. Both reactions were linear with time and protein concentration. The *N*-demethylation of ethylmorphine was estimated by measuring the amount of formaldehyde formed, according to the method of Nash.[25] The hexobarbital was estimated by measuring the remaining substrate according to the method of Cooper and Brodie,[26] as modified by Dr. M. T. Bush (personal communication); in this modification, isoamyl alcohol was omitted from the heptane to prevent extraction of norhexobarbital. The K_m, V_{max}, K_s and A_{max} were calculated with a computer program.[6]

The stoichiometry of the relationship between the amount of drug metabolized and the amount of NADPH oxidized via cytochrome P-450 was determined by the methods of Stripp *et al.*[27] These relationships can be expressed as indexes as follows: index I = V_{max} in air/NADPH-oxidation [substrate in air], index II = V_{max} in air/(NADPH-oxidation [substrate in air] −NADPH-oxidation [in air]) and index III =

$$\frac{V_{max} \text{ in air}}{(\text{NADPH-oxidation [substrate in air]}) - (\text{NADPH-oxidation [CO–O}_2\text{(9:1)]})}$$

The NADPH-oxidation [substrate in air] was measured at saturated substrate concentration, 2 mM (approx. 10 × K_m), since this rate was very close to a calculated V_{max} for NADPH-oxidation.

As discussed by Stripp *et al.*,[27] a mixed function oxidase mechanism for cytochrome P-450 systems should have an index I value of one when all of the endogenous NADPH-oxidation is catalyzed by the same cytochrome P-450 system that catalyzes the oxidation of the substrate or when the rate of NADPH-oxidation in the absence of substrate is insignificant compared with the rate in the presence of substrate. With liver microsomes, however, the value of index I is invariably less than one, because they are known to possess NADPH-oxidative systems which do not involve cytochrome P-450 and perhaps cytochrome P-450 enzymes that do not catalyze the oxidation of the substrate being studied. Index II should be one only when none of the endogenous NADPH-oxidation is mediated by the cytochrome P-450 system that catalyzes the oxidation of the substrate being studied. It will be greater than one when a significant portion of the endogenous NADPH-oxidation is mediated by the cytochrome P-450 system that catalyzes the oxidation of the substrate or when the substrate inhibits NADPH-oxidation along other pathways. It can be less than one when the substrate stimulates NADPH-oxidation via partially abortive complexes or when the formation of the product being assayed represents a minor portion of the total metabolism of the substrate. Index III should be one when all of the CO-inhibitable portion of the endogenous NADPH-oxidation is catalyzed by the cytochrome P-450 system that catalyzes the oxidation of the substrate. It will be less than one when at low substrate concentrations the rate of substrate oxidation is less than V_{max} or when an appreciable portion of the CO-inhibitable endogenous NADPH-oxidation is not mediated by the cytochrome P-450 system that catalyzes the oxidation of the substrate to the product being assayed.

Groups comprised four animals in the acute studies and three animals in the chronic studies. All the above mentioned activities and components were measured within the same day. The Student's *t*-test was used to make comparisons between the control and treated animals.

RESULTS

Binding of hexobarbital and ethylmorphine to cytochrome P-450 in female rats. The data in Table 1 demonstrate that the pretreatment of female rats with spironolactone, methyltestosterone or cortisone acetate increased the binding of hexobarbital to cytochrome P-450 when the A_{max} was calculated with respect to P-450 content, but did not alter the apparent K_s for hexobarbital.

Neither A_{max} nor the K_s of ethylmorphine was altered by any of the three steroids when administered acutely. By contrast, in the chronically treated animals, methyltestosterone and cortisone acetate increased the A_{max} with ethylmorphine. All three steroids increased the A_{max} per cytochrome P-450, but only methyltestosterone increased it by more than 20 per cent. Moreover, chronic pretreatment with spironolactone or methyltestosterone increased the apparent K_s, although the apparent K_s of the controls was apparently lowered by the chronic injections of corn oil.

Binding of hexobarbital and ethylmorphine to cytochrome P-450 in male rats. No appreciable change (< 20 per cent) (Table 2) occurred in the binding of hexobarbital or ethylmorphine per cytochrome P-450 in liver microsomes in male rats after acute or chronic pretreatment with the three steroids. The apparent decrease in A_{max} per milligram of protein after acute administration of cortisone acetate was due to a decrease in cytochrome P-450 (see Table 4). Moreover, only spironolactone increased the apparent K_s of both hexobarbital and ethylmorphine in the acutely pretreated group.

Effect of steroid pretreatment on cytochrome P-450 content, cytochrome P-450 reduction and cytochrome c reductase in female rats. The data in Table 3 demonstrate that acute pretreatment with methyltestosterone or cortisone acetate decreased the cytochrome P-450 content in female rat microsomes by about 20 per cent. However, chronic pretreatment with these steroids had no appreciable effect (< 15 per cent).

The NADPH-cytochrome *c* reductase activity was markedly increased after acute administration of spironolactone or cortisone acetate, but was increased by less than 20 per cent after the acute administration of methyltestosterone or the chronic administration of any of the steroids.

The endogenous rate of cytochrome P-450 reduction was not altered by any of the steroids. However, the rate of cytochrome P-450 reduction, expressed per unit of cytochrome P-450 in the presence of substrate, was increased by the acute administration of three steroids, although not significantly in the case of hexobarbital after methyltestosterone administration. After chronic treatment with the steroids, no appreciable changes were observed with respect to cytochrome P-450 reduction.

Effect of steroid pretreatments on cytochrome P-450 content, cytochrome P-450 reduction and cytochrome c reductase in male rats. In male rats the cytochrome P-450 content of microsomes was decreased by acute administration of cortisone acetate, but was not altered by any of the other treatments (Table 4).

On the other hand, the NADPH-cytochrome *c* reductase activity was increased by acute pretreatment with spironolactone or cortisone acetate, but not by acute pretreatment with methyltestosterone or by chronic pretreatment with any of the steroids.

The rate of cytochrome P-450 reduction per cytochrome P-450 in the presence of ethylmorphine was increased by acute treatment with either, spironolactone or cortisone acetate, but in the presence of hexobarbital it was increased only

TABLE 1. EFFECT OF STEROID PRETREATMENT OF FEMALE RATS ON BINDING OF HEXOBARBITAL AND ETHYLMORPHINE TO CYTOCHROME P-450 IN LIVER MICROSOMES

Pretreatment	Hexobarbital			Ethylmorphine		
	A_{max}* ($\times 10^3$)	A_{max}/cyt. P-450† ($\times 10$)	K_s ($\times 10^{-4}$ M)	A_{max}* ($\times 10^3$)	A_{max}/cyt. P-450† ($\times 10$)	K_s ($\times 10^{-4}$ M)
Acute‡						
Control	2·74 ± 0·17	0·41 ± 0·03	1·05 ± 0·24	4·85 ± 0·34	0·84 ± 0·08	0·58 ± 0·12
Spironolactone	5·27 ± 0·43§	1·05 ± 0·07§	0·81 ± 0·20	4·63 ± 0·27	0·91 ± 0·04	0·82 ± 0·14
Methyltestosterone	4·76 ± 0·36§	1·02 ± 0·07§	1·28 ± 0·07	5·18 ± 0·65	1·10 ± 0·09	0·45 ± 0·06
Cortisone acetate	2·97 ± 0·27	0·61 ± 0·06§	0·86 ± 0·10	4·64 ± 0·04	0·96 ± 0·03	0·48 ± 0·02
Chronic‖						
Control				4·26 ± 0·21	0·72 ± 0·01	0·28 ± 0·05
Spironolactone				5·03 ± 0·23	0·86 ± 0·01§	0·66 ± 0·13¶
Methyltestosterone				7·26 ± 0·57§	1·21 ± 0·01§	0·49 ± 0·05¶
Cortisone acetate				5·68 ± 0·34¶	0·86 ± 0·03¶	0·44 ± 0·04

* A_{max}: $O.D._{423-470\ nm}$/mg protein.
† A_{max}/cyt. P-450: $O.D._{423-470\ nm}/O.D._{450-490\ nm}$.
‡ Values in acute studies are the mean of four animals ±S. E.; dosage 100 mg/kg, i.p., twice a day for 4 days.
§ $P < 0·01$ with respect to control.
‖ Values in chronic studies are the mean of three animals ±S. E., dosage 20 mg/kg, s.c., for 4 weeks.
¶ $P < 0·05$ with respect to control.

TABLE 2. EFFECT OF STEROID PRETREATMENTS OF MALE RATS ON BINDING OF HEXOBARBITAL AND ETHYLMORPHINE TO CYTOCHROME P-450 IN LIVER MICROSOMES

Pretreatment	Hexobarbital			Ethylmorphine		
	A_{max}* ($\times 10^3$)	A_{max}/cyt. P-450† ($\times 10$)	K_s ($\times 10^{-4}$ M)	A_{max}* ($\times 10^3$)	A_{max}/cyt. P-450† ($\times 10$)	K_s ($\times 10^{-4}$ M)
Acute‡						
Control	7·38 ± 0·69	1·20 ± 0·02	0·74 ± 0·03	5·76 ± 0·61	0·93 ± 0·03	0·42 ± 0·03
Spironolactone	7·00 ± 0·58	1·27 ± 0·06	0·89 ± 0·02§	5·75 ± 0·59	1·04 ± 0·06	0·79 ± 0·07§
Methyltestosterone	8·96 ± 0·63	1·40 ± 0·05	0·83 ± 0·04	7·11 ± 0·65	1·10 ± 0·04‖	0·48 ± 0·02
Cortisone acetate	3·89 ± 0·72‖	1·22 ± 0·11	0·80 ± 0·02	3·20 ± 0·34‖	1·01 ± 0·05	0·40 ± 0·12
Chronic¶						
Control	8·75 ± 0·30	1·58 ± 0·07	0·65 ± 0·10	6·50 ± 0·27	1·16 ± 0·07	0·50 ± 0·03
Spironolactone	7·39 ± 1·45	1·37 ± 0·18	0·71 ± 0·05	6·36 ± 1·29	1·18 ± 0·06	0·67 ± 0·06
Methyltestosterone	9·18 ± 1·32	1·69 ± 0·16	0·60 ± 0·05	7·14 ± 0·46	1·32 ± 0·02	0·52 ± 0·03
Cortisone acetate	6·76 ± 1·26	1·29 ± 0·20	0·76 ± 0·12	5·44 ± 0·67	1·04 ± 0·06	0·54 ± 0·11

* A_{max}: O.D.$_{423-470\ nm}$/mg protein.
† A_{max}/cyt. P-450: O.D.$_{423-470\ nm}$/O.D.$_{450-490\ nm}$.
‡ Values in acute studies are the mean of four animals ±S. E. Dosage schedules as for Table 1.
§ $P < 0·01$ with respect to control.
‖ $P < 0·05$ with respect to control.
¶ Values in chronic studies are the mean of three animals ±S. E.

TABLE 3. EFFECT OF STEROID PRETREATMENTS OF FEMALE RATS ON CYTOCHROME P-450 REDUCTASE ACTIVITY, CYTOCHROME P-450 CONTENT AND CYTOCHROME *c* REDUCTASE ACTIVITY IN LIVER MICROSOMES

Pretreatment	Cytochrome P-450 ($O.D._{450-490\ nm}$/mg prot.)	Cytochrome P-450 reductase activity: Δ $O.D._{450\ nm}$/mg prot./min			Cytochrome P-450 reductase activity: Δ $O.D._{450\ nm}/O.D._{450-490\ nm}$/min		NADPH-cytochrome *c* reductase activity (nmoles/mg prot. × min)
		Endogenous	+ HB*	+ EM*	+ HB*	+ EM*	
Acute†							
Control	0·057 ± 0·001	0·630 ± 0·051	0·919 ± 0·091	0·771 ± 0·058	16·1 ± 1·6	13·5 ± 1·0	106 ± 3·3
Spironolactone	0·050 ± 0·002	0·767 ± 0·100	1·41 ± 0·160‡	1·64 ± 0·260‡	28·2 ± 3·2§	32·8 ± 5·2§	198 ± 9·4§
Methyltestosterone	0·046 ± 0·003§	0·571 ± 0·120	0·985 ± 0·230	1·07 ± 0·250	21·4 ± 5·0	23·2 ± 5·4‡	123 ± 5·2‡
Cortisone acetate	0·048 ± 0·001§	0·685 ± 0·070	1·34 ± 0·190	1·13 ± 0·120‡	27·9 ± 3·9§	23·6 ± 2·5§	169 ± 4·4§
Chronic‖							
Control	0·058 ± 0·001	0·536 ± 0·200		0·656 ± 0·073		11·3 ± 1·3	102 ± 4·2
Spironolactone	0·058 ± 0·001	0·508 ± 0·040		0·766 ± 0·046		13·2 ± 0·8	117 ± 1·7‡
Methyltestosterone	0·064 ± 0·004	0·578 ± 0·031		0·832 ± 0·120		13·0 ± 1·6	105 ± 2·7
Cortisone acetate	0·065 ± 0·002‡	0·653 ± 0·070		0·754 ± 0·023		11·6 ± 3·5	118 ± 4·1‡

* Hexobarbital (HB) and ethylmorphine (EM) were used at saturated concentration, 2 mM, to stimulate cytochrome P-450 reductase.
† Values in acute studies are the mean of four animals ±S. E.; Dosage schedules as for Table 1.
‡ $P < 0·05$ with respect to control.
§ $P < 0·01$ with respect to control.
‖ Values in chronic studies are the mean of three animals ±S. E.

TABLE 4. EFFECT OF STEROID PRETREATMENTS OF MALE RATS ON CYTOCHROME P-450 REDUCTASE ACTIVITY, CYTOCHROME P-450 CONTENT, AND CYTOCHROME *c* REDUCTASE ACTIVITY IN LIVER MICROSOMES

Pretreatment	Cytochrome P-450 ($O.D._{450-490\ nm}$/mg prot.)	Cytochrome P-450 reductase activity					NADPH-cytochrome *c* reductase activity (nmoles/mg prot. × min)
		Δ $O.D._{450\ nm}$/mg prot./min			Δ $O.D._{450\ nm}/O.D._{450-490\ nm}$/min		
		Endogenous	+ HB*	+ EM*	+ HB*	+ EM*	
Acute†							
Control	0·061 ± 0·006	0·738 ± 0·089	1·59 ± 0·23	1·52 ± 0·13	26·6 ± 3·8	24·9 ± 2·3	110 ± 4·9
Spironolactone	0·054 ± 0·004	0·769 ± 0·034	1·99 ± 0·39	1·91 ± 0·26	36·8 ± 7·2	35·3 ± 4·8‡	185 ± 7·6§
Methyltestosterone	0·064 ± 0·006	0·873 ± 0·023	2·14 ± 0·23	1·76 ± 0·20	33·4 ± 3·3	27·5 ± 3·1	125 ± 5·2
Cortisone acetate	0·031 ± 0·002§	0·561 ± 0·045	1·30 ± 0·17	1·21 ± 0·25	41·8 ± 5·4‡	39·0 ± 8·0‡	148 ± 7·3‡
Chronic‖							
Control	0·055 ± 0·001	0·929 ± 0·063	1·44 ± 0·27	1·50 ± 0·31	26·2 ± 4·9	27·0 ± 5·5	94·7 ± 10·5
Spironolactone	0·053 ± 0·006	0·684 ± 0·130	1·40 ± 0·27	1·25 ± 0·21	26·4 ± 4·9	23·6 ± 3·9	105 ± 6·7
Methyltestosterone	0·053 ± 0·003	0·658 ± 0·100	1·50 ± 0·32	1·31 ± 0·10	28·3 ± 6·0	24·7 ± 1·9	100 ± 4·9
Cortisone acetate	0·051 ± 0·004	0·770 ± 0·100	1·34 ± 0·25	1·25 ± 0·18	26·3 ± 4·9	24·5 ± 3·5	119 ± 10·7

* Hexobarbital (HB) and ethylmorphine (EM) were used at saturated concentration, 2 mM ,to stimulate cytochrome P-450 reductase.
† Values in acute studies are the mean of four animals ±S. E.
‡ $P < 0·05$ with respect to control.
§ $P < 0·01$ with respect to control.
‖ Values in chronic studies are the mean of three animals ±S. E.

TABLE 5. EFFECT OF STEROID PRETREATMENTS OF FEMALE RATS ON NADPH-OXIDATION, ON HEXOBARBITAL (HB) AND ETHYLMORPHINE (EM) METABOLISM

	NADPH-oxidation (nmoles/mg prot./min)				Hexobarbital metabolism		Ethylmorphine metabolism	
Pretreatment	Endogenous A	+ HB* B	+ EM* C	+ CO–O_2(9:1) D	V_{max} (nmoles/mg prot./min)	K_m (mM)	V_{max} (nmoles/mg prot./min)	K_m (mM)
Acute†								
Control	9·16 ± 0·46	10·2 ± 0·50	10·8 ± 0·43	3·13 ± 0·16	1·39 ± 0·087	0·23 ± 0·04	3·53 ± 0·18	0·80 ± 0·05
Spironolactone	11·4 ± 0·76‡	14·5 ± 1·08‡	19·0 ± 0·89§	3·49 ± 0·33	3·25 ± 0·33§	0·24 ± 0·04	12·4 ± 0·81§	0·50 ± 0·03§
Methyltestosterone	7·39 ± 0·58	10·6 ± 0·63	11·4 ± 0·98	2·72 ± 0·54	3·74 ± 0·57§	0·42 ± 0·10	5·79 ± 0·29§	0·54 ± 0·07‡
Cortisone acetate	9·74 ± 0·41	14·7 ± 0·76§	16·5 ± 0·94§	3·96 ± 0·45	2·52 ± 0·34‡	0·28 ± 0·03	6·79 ± 0·36§	0·50 ± 0·02§
Chronic‖								
Control	8·29 ± 0·69		9·40 ± 0·49	3·75 ± 0·36			2·28 ± 0·13	0·60 ± 0·05
Spironolactone	9·00 ± 1·05		10·4 ± 1·02	4·44 ± 0·32			3·99 ± 0·53‡	0·38 ± 0·00§
Methyltestosterone	8·31 ± 0·093		10·7 ± 0·71	4·48 ± 0·30			7·20 ± 1·28§	0·32 ± 0·02§
Cortisone acetate	9·26 ± 0·39		11·3 ± 0·23‡	3·30 ± 0·28			2·77 ± 0·52	0·43 ± 0·03‡

* Hexobarbital (HB) and ethylmorphine (EM) were used in a concentration of 2 mM to stimulate NADPH-oxidation.
† Values in acute studies are the mean of four animals ±S. E.
‡ $P < 0·05$ with respect to control.
§ $P < 0·01$ with respect to control.
‖ Values in chronic studies are the mean of three animals ± S. E.

after acute treatment with cortisone acetate. By contrast, chronic administration did not affect the rate of cytochrome P-450 reduction.

Effect of steroid pretreatment on NADPH-oxidation and on the metabolism of hexobarbital and ethylmorphine in female rats. Acutely administered spironolactone, methyltestosterone and cortisone acetate increased the metabolism (V_{max}) of both hexobarbital and ethylmorphine by liver microsomes from female rats (Table 5). The three steroids also produced a decrease in the K_m for ethylmorphine metabolism, but did not significantly alter the apparent K_m for hexobarbital metabolism.

By contrast, the K_m for ethylmorphine *N*-demethylation was decreased by the chronic administration of any of the three steroids, and the V_{max} was increased by the chronic administration of spironolactone or methyltestosterone but not by that of cortisone acetate.

Acute treatment with spironolactone increased both the endogenous rate of NADPH-oxidation and the rate of NADPH-oxidation in the presence of substrate. Cortisone acetate increased the rate of NADPH-oxidation in the presence of substrate, but had no effect on the endogenous rate of NADPH-oxidation. Methyltestosterone administration had no effect on the rate of NADPH-oxidation either in the presence or absence of substrate.

As expected, the values of index I for the metabolism of ethylmorphine and hexobarbital were always considerably less than one (Table 6), indicating that most of the total NADPH-oxidation in the presence of substrate was not mediated by the cytochrome P-450 system that catalyzed the oxidation of hexobarbital or ethylmorphine. But, the index I values for ethylmorphine metabolism were higher than those for hexobarbital metabolism.

On the other hand, the index II values for hexobarbital metabolism were greater than one with liver microsomes from control females, approached one with those from female rats acutely treated with spironolactone or methyltestosterone, but were less than one with liver microsomes from females treated with cortisone acetate. Since a considerable portion of the endogenous NADPH-oxidation was inhibitable by $CO–O_2$, however, the index III values for hexobarbital oxidation were considerably less than one.

By contrast, the index II values for ethylmorphine *N*-demethylation were considerably greater than one with liver microsomes from control females and females acutely treated with spironolactone or methyltestosterone, but approached one with liver microsomes from females acutely treated with cortisone acetate. With microsomes from chronically treated females, the index II values for ethylmorphine *N*-demethylation were always greater than one. The values of index III for ethylmorphine *N*-demethylation were less than one with all the groups of female rats, except the rats chronically treated with methyltestosterone. Nevertheless, the index III values for this substrate were significantly increased by acute treatment with spironolactone or methyltestosterone.

Effect of steroid pretreatments on NADPH-oxidation and on the metabolism of hexobarbital and ethylmorphine in male rats. The data in Table 7 demonstrate that acute administration of spironolactone, methyltestosterone and cortisone acetate decreased the V_{max} of hexobarbital, and methyltestosterone decreased the apparent K_m. Chronic administration of the three steroids, however, did not significantly affect either the V_{max} or the K_m for hexobarbital metabolism.

TABLE 6. CALCULATIONS OF INDEXES I–III AFTER ACUTE OR CHRONIC STEROID TREATMENT OF FEMALE RATS

Pretreatment	NADPH-oxidation (differences between columns in Table 5) B−A	C−A	B−D	C−D	Hexobarbital Index I V_{max} air / B	Index II V_{max} air / B−A	Index III V_{max} air / B−D	Ethylmorphine Index I V_{max} air / C	Index II V_{max} / C−A	Index III V_{max} air / C−D
Acute*										
Control	1·11 ± 0·32	1·67 ± 0·28	7·16 ± 0·50	7·71 ± 0·37	0·14 ± 0·010	1·8 ± 0·65	0·20 ± 0·013	0·33 ± 0·018	2·2 ± 0·41	0·46 ± 0·021
Spironolactone	3·02 ± 0·43†	7·60 ± 0·32†	11·0 ± 0·76†	15·5 ± 0·71†	0·22 ± 0·020	1·07 ± 0·14	0·31 ± 0·043	0·66 ± 0·015†	1·6 ± 0·10	0·80 ± 0·029†
Methyltestosterone	3·25 ± 0·16†	4·01 ± 0·42†	7·90 ± 0·28	8·70 ± 0·48	0·34 ± 0·050	1·1 ± 0·18	0·45 ± 0·069†	0·51 ± 0·02†	1·5 ± 0·10	0·67 ± 0·012†
Cortisone acetate	4·98 ± 0·48†	6·80 ± 0·69†	10·8 ± 1·07‡	12·6 ± 1·3†	0·17 ± 0·013	0·52 ± 0·05	0·24 ± 0·024	0·41 ± 0·015	1·01 ± 0·062‡	0·55 ± 0·038
Chronic§										
Control		1·11 ± 0·39		5·65 ± 0·75				0·22 ± 0·037	2·6 ± 1·1	0·37 ± 0·083
Spironolactone		2·49 ± 0·37		7·05 ± 0·014				0·35 ± 0·060	1·7 ± 0·39	0·60 ± 0·14
Methyltestosterone		2·57 ± 0·78		6·31 ± 0·43				0·68 ± 0·090†	3·6 ± 1·4	1·1 ± 0·16†
Cortisone acetate		2·04 ± 0·57		7·99 ± 0·48				0·26 ± 0·020	1·9 ± 0·80	0·38 ± 0·040

* Values in acute studies are the mean of four animals ±S. E.

† $P < 0·01$ with respect to control.

‡ $P < 0·05$ with respect to control.

§ Values in chronic studies are the mean of three animals ±S. E.

TABLE 7. EFFECT OF STEROID PRETREATMENTS OF MALE RATS ON NADPH-OXIDATION, ON HEXOBARBITAL (HB) AND ETHYLMORPHINE (EM) METABOLISM

	NADPH-oxidation				Hexobarbital metabolism		Ethylmorphine metabolism	
	(nmoles/mg prot./min)							
Pretreatment	Endogenous A	+ HB* B	+ EM* C	+CO–O_2(9:1) D	V_{max} (nmoles/mg prot./min)	K_m (mM)	V_{max} (nmoles/mg (prot./min	K_m (mM)
Acute†								
Control	7·89 ± 0·41	10·9 ± 0·27	12·3 ± 1·03	3·37 ± 0·28	8·79 ± 0·59	0·35 ± 0·02	8·50 ± 0·95	0·42 ± 0·08
Spironolactone	13·5 ± 0·38‡	20·8 ± 1·15‡	25·6 ± 0·81‡	4·61 ± 0·50	3·33 ± 1·11‡	0·16 ± 0·12	12·6 ± 0·63§	0·54 ± 0·08
Methyltestosterone	9·28 ± 0·49	14·7 ± 1·24§	15·5 ± 1·64	4·04 ± 0·33	6·18 ± 0·61§	0·14 ± 0·03‡	11·8 ± 1·08	0·38 ± 0·03
Cortisone acetate	9·76 ± 0·81	15·1 ± 1·71	15·9 ± 1·85	4·19 ± 0·71	6·08 ± 0·21‡	0·21 ± 0·06	8·32 ± 0·95	0·36 ± 0·01
Chronic‖								
Control	9·28 ± 0·46	13·7 ± 0·67	14·2 ± 0·95	4·50 ± 0·48	6·89 ± 1·24	0·39 ± 0·19	7·07 ± 1·07	0·31 ± 0·04
Spironolactone	9·70 ± 0·75	14·3 ± 1·42	14·6 ± 0·99	5·62 ± 0·26	3·89 ± 1·09	0·32 ± 0·03	5·26 ± 0·78	0·49 ± 0·04
Methyltestosterone	7·79 ± 0·46	11·4 ± 0·23§	12·4 ± 0·49	4·96 ± 0·62	7·37 ± 1·26	0·26 ± 0·08	7·30 ± 0·63	0·27 ± 0·04
Cortisone acetate	11·0 ± 0·23§	14·2 ± 0·45	15·6 ± 0·33	4·75 ± 0·15	6·20 ± 0·57	0·25 ± 0·11	6·38 ± 0·99	0·35 ± 0·06

* Hexobarbital (HB) and ethylmorphine (EM) were used in a concentration of 2 mM to stimulate NADPH-oxidation.
† Values in acute studies are the mean of four animals ±S. E.
‡ $P < 0·01$ with respect to control.
§ $P < 0·05$ with respect to control.
‖ Values in chronic studies are the mean of three animals ±S. E.

TABLE 8. CALCULATION OF INDEXES I-III AFTER ACUTE OR CHRONIC STEROID TREATMENT OF MALE RATS

Pretreatment	NADPH-oxidation (differences between columns in Table 7) B−A	C−A	B−D	C−D	Hexobarbital Index I V_{max} air B	Index II V_{max} air B−A	Index III V_{max} air B−D	Ethylmorphine Index I V_{max} air C	Index II V_{max} air C−A	Index III V_{max} air C−D
Acute*										
Control	2·99 ± 0·34	4·45 ± 0·71	7·50 ± 0·36	8·96 ± 0·74	0·80 ± 0·020	3·2 ± 0·33	1·2 ± 0·046	0·72 ± 0·19	2·4 ± 0·69	1·00 ± 0·14
Spironolactone	7·28 ± 1·15†	12·1 ± 0·77†	16·2 ± 1·20†	21·0 ± 0·89†	0·45 ± 0·11†	0·35 ± 0·044†	0·15 ± 0·014†	0·49 ± 0·011†	1·05 ± 0·033‡	0·61 ± 0·025‡
Methyltestosterone	5·40 ± 0·88‡	6·32 ± 1·08	10·6 ± 0·94	11·4 ± 1·03	0·51 ± 0·060†	1·3 ± 0·18†	0·59 ± 0·045†	0·76 ± 0·028	2·04 ± 0·19	1·04 ± 0·050
Cortisone acetate	5·36 ± 0·78	6·16 ± 0·95	11·7 ± 2·20	11·7 ± 2·20	0·42 ± 0·14	1·2 ± 0·16†	0·59 ± 0·091†	0·52 ± 0·0080	1·4 ± 0·13	0·72 ± 0·046
Chronic§										
Control	4·37 ± 0·46	4·90 ± 0·50	9·15 ± 0·60	9·70 ± 0·50	0·50 ± 0·030	1·6 ± 0·020	0·75 ± 0·026	0·49 ± 0·043	1·4 ± 0·080	0·73 ± 0·075
Spironolactone	4·23 ± 0·67	4·86 ± 1·1	8·31 ± 1·5	8·93 ± 0·78	0·27 ± 0·0007†	0·93 ± 0·066†	0·48 ± 0·055‡	0·36 ± 0·032	1·2 ± 0·34	0·58 ± 0·039
Methyltestosterone	3·57 ± 0·44	4·57 ± 0·48	7·07 ± 0·43	8·07 ± 0·43	0·65 ± 0·11	2·2 ± 0·55	1·03 ± 0·12	0·58 ± 0·029	1·6 ± 0·18	0·91 ± 0·075
Cortisone acetate	3·17 ± 0·55	4·53 ± 0·56	9·47 ± 0·45	10·8 ± 0·19	0·43 ± 0·031	2·06 ± 0·32	0·65 ± 0·034	0·40 ± 0·054	1·4 ± 0·22	0·58 ± 0·081

* Values in acute studies are the mean of four animals ± S. E.

† $P < 0·01$ with respect to control.

‡ $P < 0·05$ with respect to control.

§ Values in chronic studies are the mean of three animals ± S. E.

By contrast, the rate of metabolism of ethylmorphine was increased by the acute administration of spironolactone, but was not significantly affected by the acute administration of the other steroids nor by the chronic administration of any of the steroids.

Acute administration with the three steroids tended to increase NADPH-oxidation both in the presence and absence of either substrate, but this increase was appreciable only with spironolactone. By contrast, in the chronic study, methyltestosterone decreased the NADPH-oxidation in the presence of hexobarbital, whereas the other steroids were without appreciable effect.

Although the index I values for the metabolism of hexobarbital and ethylmorphine by liver microsomes from the various treatment groups of male rats were invariably less than one (Table 8), the values were generally higher with males than with females (cf. Tables 6 and 8).

The index II values for hexobarbital metabolism were greater than one with control males (Table 8), less than one with males acutely treated with spironolactone, and approached one with males acutely treated with methyltestosterone or cortisone acetate. With the chronic treatment groups, the index II values for hexobarbital metabolism were greater than one with all groups, except that treated with spironolactone. Moreover, the index III values for hexobarbital approached one in the control groups and in the group chronically treated with methyltestosterone, but were considerably less than one in the other groups.

By contrast, the index II values for ethylmorphine *N*-demethylation approached one with the groups of males receiving spironolactone either acutely or chronically, but values with the other groups were greater than one. Moreover, the index III values for ethylmorphine *N*-demethylation approached one with the groups of males serving as controls or treated with methyltestosterone, but the value was significantly decreased in the group of males receiving spironolactone acutely.

TABLE 9. EFFECT OF CHRONIC STEROID PRETREATMENT OF MALE AND FEMALE RATS ON THE WEIGHT OF THE SEMINAL VESICLES AND UTERI RESPECTIVELY

Pretreatment	Females (g uterus/100 g body wt)	Males (g seminal vesicles/100 g body wt)
Control	0·54 ± 0·035	0·14 ± 0·006
Spironolactone	0·47 ± 0·007	0·08 ± 0·010*
Methyltestosterone	0·62 ± 0·012	0·46 ± 0·060*
Cortisone acetate	0·79 ± 0·13	0·23 ± 0·033†

* $P < 0·01$ with respect to control.
† $P < 0·05$ with respect to control.

Effect of chronic steroid pretreatment of male and female rats on the weight of the seminal vesicles and uteri respectively. The data in Table 9 demonstrate that in the females the ratio of the uterus to body weight is not significantly altered by any of the steroids. However, the ratio of seminal vesicle to body weight is decreased by spironolactone and increased by methyltestosterone and cortisone acetate.

DISCUSSION

Despite the fact that chronic administration of the three steroids to male rats resulted in significant changes in the weight of the seminal vesicles (Table 9), there were relatively few changes in the metabolism of hexobarbital or ethylmorphine or in the various components of the mixed function oxidase system (Tables 2, 4 and 7). Spironolactone showed an anti-androgenic effect (Table 9), which is in accord with previous findings.[19,28] The reason for the overall lack of effect of chronic treatment might be that mature male rats possess sufficient androgens and glucocorticoids to evoke optimal effects on drug metabolism and that a chronic administration of a relatively small dose of either of these steroids will therefore not be able to evoke any further effect.

It might be expected that chronic administration of methyltestosterone to female rats increases ethylmorphine metabolism, since this steroid has been shown to enhance hexobarbital hydroxylation and the demethylation of monomethyl-4-amino-antipyrine.[20] Accordingly, not only methyltestosterone but also spironolactone significantly increased the V_{max} for ethylmorphine, while cortisone acetate showed no effect. The increased drug metabolism in females might be due to a number of changes, which would tend to increase the V_{max} (Table 5), namely the increased A_{max} per cytochrome P-450 (Table 1) or the increased NADPH-cytochrome *c* reductase activity (Table 3). Since methyltestosterone increased the rate of metabolism without increasing NADPH-cytochrome *c* reductase activity, it appeared that methyltestosterone might exert its effect mainly by causing a qualitative change in cytochrome P-450. In support of this theory, we found (unpublished results) that the ratio 455–490/430–490 nm in the ethylisocyanide difference spectrum at pH 7·4 was decreased from 0·98 to 0·50 after chronic treatment with methyltestosterone (thus approaching the value in male rats),[29] but was not altered after treatment with spironolactone or cortisone acetate.

By contrast, the acute administration of the steroids to rats caused profound changes in the cytochrome P-450 system in liver microsomes of both sexes. In female rats both the rate of hexobarbital hydroxylation and ethylmorphine *N*-demethylation was significantly increased by all three steroids, but spironolactone showed a much greater effect on ethylmorphine metabolism than did methyltestosterone or cortisone acetate. It is difficult to ascertain which of the aspects studied might account for the changes in the metabolic activity, especially because these aspects were not equally affected with respect to the two substrates. In the animals treated with spironolactone or cortisone acetate, increases in ethylmorphine *N*-demethylation might have been due to increases in NADPH-cytochrome *c* reductase and thus to increases in the rate of reduction of the cytochrome P-450–substrate complex (Table 3), activities which have been linked with sex-dependent ethylmorphine metabolism.[6,11] Moreover, hexobarbital hydroxylation might have been increased due to increased formation of cytochrome P-450–substrate complex, as indicated by A_{max} per cytochrome P-450 (Table 1), as well as to increases in NADPH-cytochrome *c* reductase. This is in agreement with the findings of Schenkman *et al.*,[7] who showed a correlation between A_{max} and hexobarbital metabolism in male and female rats. Thus, the increased A_{max} might, at least in the case of spironolactone or cortisone acetate, have given rise to the rate of reduction of cytochrome P-450–substrate complex (Table 3), which together with the increased NADPH-cytochrome *c* reductase activity (Table 5) would result in an increased V_{max}. Methyltestosterone, however, seemed to act through a different mechanism, since only the A_{max} with

hexobarbital was increased. It was especially noteworthy, however, that all three steroids increased the V_{max} values without increasing the amount of cytochrome P-450; in fact, in some instances they caused a decrease (Table 3). Moreover, we found that the ratio 455–490/430–490 nm in the ethylisocyanide difference spectrum was not affected by acute treatment with any of the steroids (unpublished results).

The acute treatment of male rats gave quite different results. Decreases were observed in the rates of hexobarbital hydroxylation after treatment with any of the steroids, but especially after spironolactone administration, while an increase in ethylmorphine *N*-demethylation was found only after spironolactone (Table 7). These findings with spironolactone are in agreement with previous results,[19] but they are nevertheless difficult to explain. After administration of spironolactone, the rates of reduction of cytochrome *c* and the cytochrome P-450–hexobarbital complex were not decreased. Moreover, after methyltestosterone the A_{max} of the cytochrome P-450–hexobarbital complex and its reduction rate tended to increase, yet the V_{max} was decreased.

Owing to the difficulties in relating changes in drug metabolism and especially in hexobarbital metabolism to changes in the various components of the cytochrome P-450 system, it seemed possible that other factors may also be important in determining the rate of drug metabolism. Indeed studies on the relationship between NADPH-oxidation and substrate metabolism have revealed surprising sex differences. With liver microsomes from control female rats, the values of neither index II nor III approached one for the metabolism of either ethylmorphine or hexobarbital (Table 6), suggesting that considerable endogenous NADPH-oxidation was mediated by the hexobarbital or ethylmorphine-oxidation system, but that most of the endogenous NADPH-oxidation inhibited by CO was not associated with the metabolism of either substrate. With liver microsomes from male rats, however, the index II values for hexobarbital and ethylmorphine metabolism were considerably greater than one, whereas the index III values were close to one, indicating that a considerable portion of the endogenous NADPH-oxidation was mediated by the drug-metabolizing systems and that virtually all of the CO-inhibitable endogenous NADPH-oxidation was mediated by these systems. Since V_{max} values for hexobarbital oxidation were used in these calculations, these findings are consistent with the view that the cytochrome P-450 from liver microsomes in female rats differs qualitatively from that of liver microsomes in male rats.

Moreover, only a relatively small portion of the cytochrome P-450 in liver microsomes from female rats can function for the metabolism of either hexobarbital or ethylmorphine. These results are thus consistent with the findings of Schenkman *et al.*[7] that there is a sex difference in A_{max} values for hexobarbital (see also Table 1 and 2) and those of Stripp *et al.*[30] that there is a sex difference in the extinction coefficient of cytochrome P-450.

The acute treatment of female rats with spironolactone tended to decrease the index II value for hexobarbital toward one, and had little effect on its index III value, but the treatment in male rats decreases these values to below one. Thus after the treatment of male rats with this steroid, hexobarbital stimulated NADPH-oxidation to a greater extent than could be accounted for by its metabolism (cf. Table 7, V_{max} column with Table 8, B—A column). Similarly, treatment of female rats with cortisone acetate decreases both index II and III to much less than one. These findings suggest

that hexobarbital combines with cytochrome P-450 to form a complex which is rapidly reduced, but that during its reoxidation only some of the complex leads to the formation of oxidized hexobarbital and the rest is abortive. Indeed, the formation of abortive complexes may account for the fact that spironolactone treatment of male rats decreases the metabolism of hexobarbital by liver microsomes, even though the various components of the cytochrome P-450 systems either are increased or remain relatively unchanged.

The values of indexes II and III for ethylmorphine are higher than those for hexobarbital with liver microsomes from control female rats and from female rats acutely treated with any of the three steroids (Table 6). Thus, a greater portion of the endogenous NADPH-oxidation apparently is mediated by the ethylmorphine *N*-demethylation system than it is by the hexobarbital oxidation. These findings thus suggest that a greater portion of the cytochrome P-450 in liver microsomes of female rats is utilized in the metabolism of ethylmorphine than it is in the metabolism of hexobarbital, even though both substrates cause type I spectral changes with liver microsomes. Moreover, the finding that spironolactone treatment of male rats decreases both index II and III for ethylmorphine raises the possibility that this treatment also leads to the formation of abortive cytochrome P-450–ethylmorphine complexes in addition to abortive cytochrome P-450–hexobarbital complexes, but that the effect is less marked with this substrate and thus does not become obvious.

These studies have thus provided evidence that the rate of drug metabolism is determined by factors which cannot be directly related to changes in cytochrome P-450 content, the magnitude of type I spectral changes or the NADPH-cytochrome *c* reductase activity. They further demonstrate that it is a mistake to extrapolate the findings obtained with one type I substrate to other type I substrates.

The data also suggest that male rats possess sufficient androgens and glucocorticoids to evoke maximal effects on the metabolism of hexobarbital and ethylmorphine. The accelerated flux of electrons through the mixed function oxidase system, as indicated by the increased rate of reduction of cytochrome *c* and cytochrome P-450–substrate complex and oxidation of NADPH in presence of substrate observed after acute treatment with high doses of steroids, is actually accompanied by a decreased formation of metabolites. These steroids in male rats may then result in an uncoupling of the substrate from the electron transport system at an undetermined point, for instance, at the formation of the active oxygen intermediate. In female rats, on the other hand, all three steroids stimulate the drug metabolism. The reason for these effects, however, is not clear at present because of lack of knowledge about the steroids as endogenous substrates and modifiers of the mixed function oxidation system in liver microsomes.

REFERENCES

1. J. A. Castro, F. E. Greene, P. Gigon, H. Sasame and J. R. Gillette, *Biochem. Pharmac.* **19**, 2461 (1970).
2. R. Kato, A. Takahashi, K. Onoda and Y. Omori, *Endocr. Jap.* **17**, 207 (1970).
3. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 285 (1965).
4. S. Tehu and N. Yago, *J. biol. Chem.* **65**, 597 (1969).
5. H. Remmer, *Naturwissenschaften* **45**, 522 (1958).
6. D. S. Davies, P. L. Gigon and J. R. Gillette, *Biochem. Pharmac.* **17**, 1865 (1968).
7. J. B. Schenkman, I. Frey, H. Remmer and R. W. Estabrook, *Molec. Pharmac.* **3**, 516 (1967).
8. R. Kato and K. Onoda, *Biochem. Pharmac.* **19**, 1649 (1970).

9. R. KATO, H. TAKAHASHI and Y. OMORI, *Endocr. jap.* **16,** 653 (1969).
10. P. L. GIGON, T. E. GRAM and J. R. GILLETTE, *Biochem. biophys. Res. Commun.* **31,** 558 (1968).
11. P. L. GIGON, T. E. GRAM and J. R. GILLETTE, *Molec. Pharmac.* **5,** 109 (1969).
12. D. S. DAVIES, P. L. GIGON and J. R. GILLETTE, *Life Sci.* **8,** 85 (1969).
13. G. P. QUINN, J. AXELROD and B. B. BRODIE, *Biochem. Pharmac.* **1,** 152 (1958).
14. H. REMMER, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **236,** 7 (1959).
15. F. M. RADZIALOWSKI and W. F. BOUSQUET, *J. Pharmac. exp. Ther.* **163,** 229 (1968).
16. W. F. BOUSQUET, B. D. RUPE and T. S. MIYA, *J. Pharmac. exp. Ther.* **147,** 376 (1965).
17. R. E. STIZEL and R. L. FURNER, *Biochem. Pharmac.* **16,** 1489 (1967).
18. B. SOLYMOSS, H. G. CLASSEN and S. VARGA, *Proc. Soc. exp. Biol. Med.* **132,** 940 (1969).
19. B. STRIPP, M. E. HAMRICK, N. G. ZAMPAGLIONE and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **176,** 766 (1971).
20. J. BOOTH and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **137,** 374 (1962).
21. T. OMURA and R. SATO, *J. biol. Chem.* **239,** 2370 (1968).
22. H. REMMER, J. B. SCHENKMAN, R. W. ESTABROOK, H. A. SASAME and J. R. GILLETTE, *Molec. Pharmac.* **2,** 187 (1966).
23. A. G. GORNALL, C. J. BARDAWILL and M. M. DAVID, *J. biol. Chem.* **177,** 751 (1949).
24. A. PHILLIPS and R. LANGDON, *J. biol. Chem.* **237,** 2652 (1962).
25. I. NASH, *Biochem. J.* **55,** 416 (1953).
26. J. R. COOPER and B. B. BRODIE, *J. Pharmac. exp. Ther.* **114,** 409 (1955).
27. B. STRIPP, N. ZAMPAGLIONE, M. HAMRICK and J. R. GILLETTE, *Molec. Pharmac.* **8,** 189 (1972).
28. S. L. STEELMAN, J. R. BROOKS, E. R. MOYAN and D. J. PATANELLI, *Steroids* **14,** 449 (1969).
29. S. A. M. EL DEFRANY, *Studies on the Sex Dependent Difference in Drug Metabolism in the Rat,* Ph.D. Thesis. University of Minnesota, 68–7306, University Microfilms, Ann Arbor, Mich. (1967).
30. B. STRIPP, F. E. GREENE and J. R. GILLETTE, *Pharmacology* **6,** 56 (1971).