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EFFECTS OF CONTRACEPTIVE AGENTS ON DRUG METABOLISM IN VARIOUS ANIMAL SPECIES

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1 The effect on liver microsomal enzyme activity of three steroid contraceptive drug (SCD) combinations was compared in rats, mice and guinea-pigs. Lynestrenol plus mestranol, norethisterone plus mestranol and norethynodrel plus mestranol were given orally for 4 consecutive days (acute treatment) or 30 days (chronic treatment) at various doses eliciting an experimentally controlled antifertility activity which varied in its extent.

2 In rats and mice all the combined treatments (with the exception of norethynodrel plus mestranol in mice) were active as inducers of liver microsomal enzymes. This induction seems to be mediated mainly by the progestogenic compounds. Oestrogens showed a very poor effect bordering on significance only in a few cases.

3 No effect on liver microsomal protein or cytochrome P 450 concentration was obtained after treatment with doses capable of increasing the microsomal enzyme activity.

4 The activity of the liver microsomal enzymes did not appear to be reduced immediately (2 h) after the last administration of the SCD given during 4 or 30 days.

5 Contraceptive treatments at doses capable of eliciting complete antifertility activity were inactive on liver microsomal enzyme activity in guinea-pigs.

Introduction

It has been shown that both endogenous and exogenous steroids are metabolized in the liver endoplasmic reticulum by the same enzyme system responsible for the metabolism of foreign compounds (Conney, 1967). There are also extensive reports on the effects exerted by steroids on liver microsomal enzyme activity and on the interference by these hormones with the metabolism of some drugs. Particular attention was given in this respect to the steroid contraceptive drugs (SCD) in view of their widespread use together with other drugs in human therapy. Progesterone (Juchau & Fouts, 1966; Tephly & Mannering, 1968; Soyka & Long, 1972) and progestogen compounds (Soyka & Deckert, 1974; Tüttenberg, Hüthwohl, Kahl & Kahl, 1974) have been found to be competitive inhibitors for substrates metabolized by the mixed oxidases *in vitro*. More contradictory results have been obtained *in vivo*. Previous studies from this laboratory (Jori, Bianchetti & Prestini, 1969) indicated that chronic treatment with steroid contraceptives did not block, but rather increase the activity of liver microsomal enzymes in rats. Similar inducing effects were obtained by Juchau & Fouts (1966) and Rümke & Noordhoek (1969).

An increase in the metabolism of barbiturates after oestrogen and progestogen contraceptive treatment was also found in mice (Blackham & Spencer, 1969; Garg & Ahmad, 1974).

At variance with these results, Freudenthal & Amerson (1974) failed to show any induction after treatment with synthetic progestogens but on the contrary, found a weak inhibition of microsomal drug metabolism. An impairment of drug metabolism was also reported in women taking oral steroid contraceptives (Crawford & Rudofsky, 1966; O'Malley, Stevenson & Crroks, 1972).

It was therefore of interest to investigate the effect of some of the more widely used contraceptive drugs on liver microsomal enzyme activities under experimental conditions which reproduced the pattern of human use. Therefore, we have administered chronically for periods covering one or more oestrous cycles, combined oestrogens and progestogens in the ratios used for contraceptive medication and in doses able to produce an experimentally controlled antifertility activity in several animal species.

Methods

Animals

Female Charles River rats weighing 220 ± 10 g, female CD_1 mice weighing 25 ± 3 g, and female PIR bright/Z guinea-pigs weighing 350 ± 50 g were used. The rats and mice were housed in makrolon cages (6 per cage) and the guinea-pigs in steel rod cages (5 per cage). The animals were kept at room temperature (22°C) with relative humidity of 60% and with a controlled light cycle of 12 h (6 h 30 min to 18 h 30 min). Food and water were given *ad libitum*.

Drugs

The following drug combinations were used: norethynodrel plus mestranol, norethisterone plus mestranol and lynestrenol plus mestranol.

The drugs were dissolved in corn oil, given orally for a period of 4 days (acute treatment) or 30 days (chronic treatment). In the case of guinea-pigs treatment lasted 32 days which is the length of two oestrous cycles.

The doses used in each treatment group are shown in the tables. Controls received a corresponding amount of corn oil. The oestrous cycle was controlled by means of vaginal smears. Animals were killed by decapitation 2 h (at 17 h 00 min) or 18 h (at 9 h 00 min) after the last SCD administration. The livers were removed and immediately frozen and stored at −20°C.

Enzymatic activity was measured on the 9000 *g* supernatant fraction using the experimental conditions described by Kato & Takanaka (1967).

The supernatant was incubated with glucose-6-phosphate (50 μmol), nicotinamide (50 μmol), NADP (1.5 μmol), $MgCl_2$ (25 μmol), sodium phosphate buffer pH 7.4 (0.2 M) and substrates (aniline (5 μmol) *p*-nitro-anisole (1,5 μmol) and aminopyrine (5 μmol)) to a total mixture volume of 5 ml. The matabolites formed, *p*-aminophenol, *p*-nitrophenol and 4-amino antipyrine respectively, were determined by the method of Gilbert & Golberg (1965).

Liver microsomes, used for the determination of proteins by the method of Lowry, Rosenbrough, Farr & Randall, 1951 and of cytochrome P 450 by the method of Omura & Sato (1964), were prepared by Ca^{2+} precipitation by the method described by Schenkman & Cinti (1972).

The antifertility effect was measured by a bioassay as follows:

Rats: in the acute experiments (4 days = 1 oestrous cycle) the first dose of the test compound was given on the morning of pro-oestrus. At 16 h 00 min on the same day 2 females were caged with 1 fertile male. In the chronic experiments (30 days) female rats were caged with males on the 26th day of treatment. In both types of experiments, fertilization was confirmed by the presence of sperm in the vaginal smears. At autopsy, 10 days after the end of treatment, the uterine horns were examined for the number of implantation sites.

Mice: the same procedure as used with rats was followed for mice with the exception that no attempt was made to control their oestrous cycle or to confirm fertilitization by the presence of sperm in the vaginal smears.

Guinea pigs: female adult guinea-pigs (65 days old) were treated with the contraceptive agents for 33 days in order to cover two oestrous cycles. Treatment was started 10 days after checking the opening of the vaginal membrane. On the second day of treatment 2 females were caged with 1 male and left together for the whole 33 days period. No attempt was made to confirm fertilization by the presence of sperm in vaginal smears. At autopsy, 25 days after the last administration, uterine horns were examined and the number of implantation sites recorded.

Statistical analysis of the averages was carried out by Student's *t* test.

Results

Antifertility activity of the contraceptive drugs in rats, mice and guinea-pigs

In Table 1 the antifertility efficacy of the combined treatment with lynestrenol + mestranol, norethisterone + mestranol and norethynodrel + mestranol in the three animal species studied, is reported. These data are necessary to establish the significance of the steroid contraceptive drug dosages on the liver microsomal enzyme activity. It should be noted that the efficacy of the contraceptives with the exception of norethynodrel + mestranol, is similar in rats and mice after an acute treatment covering one oestrous cycle (4 days). In rats no significant difference appears between the chronic (30 days) and acute treatment; in mice the results indicate a higher activity of the combinations given chronically. In guinea-pigs all the contraceptive drugs tested appeared to be more effective than in rats and mice, although the 32 days chronic treatment covers only two complete oestrous cycles.

Liver microsomal activity in rats and mice during the oestrous cycle

Vaginal smears were examined daily from untreated rats and mice for three consecutive oestrous cycles (12 days). In the experiments with the mice 2 males were

confined in a wire basket in the cage containing 10 females to obtain more regular oestrous cycles (Whitten, 1957; Whitten, 1958). Animals with anomalous rhythms were eliminated, and those with regular four-day cycles were divided into three groups of 10 animals each and killed in pro-oestrus, oestrus and dioestrus respectively. No differences were observed during the various phases of the cycle on the metabolic activity of the liver microsomal enzymes *in vitro* or on the levels of cytochrome P 450 (nmol/mg protein 0.510 ± 0.005; 0.520 ± 0.008; 0.460 ± 0.054 in pro-oestrus, oestrus and dioestrus respectively in the rat). Therefore, the effect of the treatment with contraceptive steroids was compared in subsequent experiments with the activity of a control group without taking into consideration the oestrous state of the animals.

Effects of treatment with contraceptive drugs on liver microsomal enzyme activity in rats

Acute treatment. The effect of a short period of treatment (4 days), corresponding to one oestrous cycle, with a large dose of lynestrenol (5 mg/kg) plus mestranol (0.3 mg/kg) or of norethisterone (4 mg/kg) plus mestranol (0.2 mg/kg) was studied in rats. Results of these experiments are shown in Table 2. No significant effect on microsomal enzyme activity was obtained in rats killed 18 h after the last administration of the first SCD combination, but treatment with the second combination in the same experimental conditions significantly increased the enzyme activity for the three substrates utilized.

Table 1 Antifertility effects of contraceptive drugs on rats, mice and guinea-pigs

Treatment *(mg/kg per day, orally)*		*Number of implantation sites (% of controls)* *Rat*		*Mouse*		*Guinea-pig*
		Days of treatment				
		4	30	4	30	32
Lynestrenol + mestranol	(5) (0.3)	0	0	0	0	0
Lynestrenol + mestranol	(2.5) (0.15)	45	33	6	0	0
Lynestrenol + mestranol	(1.25) (0.075)	45	33	32	17.5	0
Lynestrenol + mestranol	(0.625) (0.037)	60	68	—	—	10
Norethisterone + mestranol	(4) (0.2)	1	0	9	6	0
Norethisterone + mestranol	(2) (0.1)	25	10	27	9	0
Norethisterone + mestranol	(1) (0.05)	39	37	48	10	0
Norethynodrel + mestranol	(4) (0.006)	5	10	5	0	0
Norethynodrel + mestranol	(2) (0.03)	31	44	8	1	0
Norethynodrel + mestranol	(1) (0.015)	56	50	6	0	0

Groups of 15–20 animals were used for each dose. In control animals the following values were obtained: rats, pregnancy rate $86.2\% \pm 5$—mean implantation sites 10.5 ± 0.4; mice, pregnancy rate $76.3\% \pm 4$—mean implantation sites 7.6 ± 0.6; guinea-pigs, pregnancy rate $67.7\% \pm 7$—mean implantation sites 2.2 ± 0.2.

Chronic treatment. Table 3 shows that a significant increase in the enzymatic activity for all the substrates used appears in each group of rats after 30-day treatment with the three combinations when the animals were killed 18 h after the last SCD administration. A dose-effect relationship is present for the combination of lynestrenol plus mestranol which was tested at three different doses. The treatments corresponding to groups II, V and VI produced a comparable decrease of fertility in rats.

Effects of treatment 2 h before the animals were killed. Results obtained in rats killed 2 h after the last administration at the end of 4 or 30 day treatment with the highest dose of the SCD combinations showed that such treatments did not modify the activity of the liver microsomal enzymes in comparison with the activity of these enzymes in control rats. These results, which are not given here in detail, indicate that, at least in rats, the inductive effect on the liver microsomal enzyme activity produced by these contraceptive agents does not appear during the first 2 h after the treatment.

Effects of treatment with contraceptive drugs on liver microsomal enzyme activity in mice

Acute treatment. The effects of acute treatment (4 days) corresponding to one oestrous cycle with several doses of the three SCDs, was studied in mice. The data in Table 4 show that lynestrenol plus mestranol and norethisterone plus mestranol are very active

Table 2 Effects of acute treatment with contraceptive drugs on liver microsomal activity in rats

Treatment (mg/kg per day, orally for 4 days)		*Enzymatic activity (nmol g^{-1} h^{-1} ± s.e.)*		
		Aminopyrine	*Aniline*	*pNO_2 Anisole*
Controls		168 ± 15	683 ± 36	532 ± 25
Lynestrenol + mestranol	(5) (0.3)	189 ± 13	606 ± 33	498 ± 27
Norethisterone + mestranol	(4) (0.2)	326 ± 20*	787 ± 31*	695 ± 24*

Each figure represents the mean value for 8 animals. Rats were killed 18 h after the 4th day of treatment. Enzymatic activity is represented by the metabolites formed, 4-aminoantipyrine, *p*-NH_2 phenol, and *p*-NO_2-phenol, respectively from the 9000 *g* supernatant fraction, corresponding to 640 mg of fresh liver.
* $P < 0.01$ versus controls.

Table 3 Effects of chronic treatment with contraceptive drugs at various dose levels on liver microsomal enzyme activity in rats

Group	*No. of animals*	*Treatment (mg/kg per day) orally for 30 days)*		*Enzymatic activity (nmol g^{-1} h^{-1} ± s.e.)* *Aminopyrine*	*Aniline*	pNO_2 *Anisole*
I	10	Controls		230 ± 25	667 ± 68	615 ± 46
II	10	Lynestrenol + mestranol	(1.25) (0.075)	379 ± 29*	829 ± 57	803 ± 34*
III	10	Lynestrenol + mestranol	(2.5) (0.15)	485 ± 33*	977 ± 35*	876 ± 34*
IV	10	Lynestrenol + mestranol	(5) (0.30)	530 ± 24*	1100 ± 41*	892 ± 28*
V	7	Norethisterone + mestranol	(2) (0.1)	435 ± 36*	1233 ± 36*	794 ± 38*
VI	7	Norethynodrel + mestranol	(2) (0.03)	324 ± 20*	1081 ± 49*	738 ± 27*

Animals were killed 18 h after 30th day of treatment. The experimental conditions were as described in Table 2.
* $P < 0.01$ versus controls.

Table 4 Effect of acute treatment with contraceptive drugs on liver microsomal enzyme activity in mice

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Treatment (mg/kg orally) daily × 4 days		*Enzymatic activity (nmol g^{-1} h^{-1} ± s.e.)* *Aminopyrine*	*Aniline*	*pNO_2Anisole*
Controls		392 ± 21	626 ± 22	821 ± 16
Lynestrenol + mestranol	(5) (0.3)	707 ± 27*	1034 ± 112*	1337 ± 34*
Lynestrenol + mestranol	(1.25) (0.075)	609 ± 18*	885 ± 67*	1181 ± 4*
Norethisterone + mestranol	(4) (0.2)	686 ± 34*	828 ± 33*	1069 ± 58*
Norethisterone + mestranol	(2) (0.1)	608 ± 39*	863 ± 22*	1092 ± 30*
Norethisterone + mestranol	(1) (0.05)	473 ± 21**	709 ± 21**	922 ± 20**
Norethynodrel + mestranol	(4) (0.06)	335 ± 9	511 ± 23	721 ± 42
Norethynodrel + mestranol	(2) (0.03)	432 ± 25	672 ± 28	812 ± 29
Norethynodrel + mestranol	(1) (0.015)	389 ± 57	567 ± 66	713 ± 75

Each figure is the mean of 5 determinations. The animals were killed 18 h after the 4th administration. Experimental conditions are described in Table 2.
* $P < 0.01$ versus controls; ** $P < 0.05$ versus controls.

Table 5 Effect of mestranol and lynestrenol on microsomal enzyme activity in rats and mice

Animal species	*Treatment (mg/kg per day, orally)*		*No. of days*	*Enzymatic activity (nmol g^{-1} h^{-1} ± s.e.)* *Aminopyrine*	*Aniline*	*pNO_2Anisole*
Rat	Controls			243 ± 11	790 ± 62	501 ± 40
	Mestranol	(0.3)	30	282 ± 20	837 ± 48	691 ± 51*
	Lynestrenol	(5)	30	345 ± 23*	1135 ± 126*	819 ± 36*
	Lynestrenol + mestranol	(5) (0.3)	30	530 ± 24*	1100 ± 41*	892 ± 28*
Mouse	Controls			416 ± 20	916 ± 34	1039 ± 40
	Mestranol	(0.3)	4	543 ± 14*	774 ± 89	1033 ± 35
	Lynestrenol	(5)	4	593 ± 18*	916 ± 69**	1303 ± 86**
	Lynestrenol + mestranol	(5) (0.3)	4	707 ± 27*	1034 ± 112*	1337 ± 34*

Each figure is the mean from at least 6 animals. Animals were killed 18 h after the last treatment. Experimental conditions as described in Table 2.
* $P < 0.01$ versus controls; ** $P < 0.05$ versus controls.

Table 6 Effect of contraceptive drugs on liver microsomal proteins and cytochrome P 450

Animal species	*No. of animals per group*	*Treatment (mg/kg, orally)*		*No. of days*	*Final body wt. (g ± s.e.)*	*Body wt. change (g ± s.e.)*	*Liver wt. (g/100 g body wt.)*	*Microsomal protein (mg/g ± s.e.)*	*Cytochrome P 450 (nmol/mg protein)*
Rat	10	Controls			244 ± 5	+41 ± 5	4.3 ± 0.02	19 ± 0.6	0.410 ± 0.03
	10	Lynestrenol + mestranol	(1.25) (0.075)	30	221 ± 2	+36 ± 2	4.4 ± 0.17	22 ± 1.4	0.424 ± 0.04
	10	Lynestrenol + mestranol	(2.5) (0.15)	30	225 ± 2	+34 ± 5	4.5 ± 0.13	21 ± 1.2	0.410 + 0.04
	5	Lynestrenol + mestranol	(5) (0.3)	30	204 ± 3	+17 ± 3*	4.8 ± 0.19	24 ± 2.7	0.484 + 0.04
Mouse	5	Controls		4	25.5 ± 0.4	+0.6	5.5 ± 0.14	16 ± 0.5	0.305 ± 0.03
	5	Lynestrenol + mestranol	(5) (0.3)	4	28.3 ± 0.7	+1.1	5.9 ± 0.20	15 ± 0.4	0.390 ± 0.03

Body weight was measured before the first treatment and immediately before animals were killed, 18 h after the last treatment.
* $P < 0.01$ versus controls

inducers of microsomal enzymes even at the lowest doses tested. This effect appears in mice as in rats, at doses that do not exert a complete antifertility action.

On the other hand, combined treatment with norethynodrel and mestranol did not affect microsomal activity in mice. This SCD combination was also inactive at effective antifertility doses when given for a period of 30 days.

Comparison between the effects of oestrogen and progestogenic compounds on microsomal enzyme activity in rats and mice

The following experiment was performed to test whether the inducing effect exerted by the contraceptive combination is dependent on the oestrogen or progestogenic compounds or on both. Rats and mice were treated for 30 and 4 days respectively, with lynestrenol and mestranol at the highest dose (5 and 0.3 mg/kg orally) used in the combined treatment in the previous experiments. The results are given in Table 5 and show that a significant increase in drug metabolism *in vitro* was always obtained when lynestrenol was present in the treatment. The effect of the mestranol is lacking or at the border line of significance. The data reported here suggest that the induction produced by the drug combination of lynestrenol plus mestranol is probably dependent only on the activity of the progestinic compound, lynestrenol as far as the aromatic hydroxylation and *O*-demethylation reactions are concerned. For the aminopyrine demethylation a synergistic effect of the two steroids cannot be excluded.

Effects of SCDs on liver weight, microsomal proteins and cytochrome P 450 concentration in rats and mice

After combined treatments with lynestrenol and mestranol at doses capable of producing a significant induction in the liver microsomal enzyme activity of rats and mice, neither liver enlargement, nor an increase in liver micosomal proteins and cytochrome P 450 concentration were observed. The only significant observation was a reduction in body weight gain in the rats treated daily for 30 days with the highest dose of the combined treatment (Table 6).

Effects of chronic treatment with SCDs on liver microsomal enzyme activity in guinea-pigs

The effect of the contraceptive drugs on liver microsomal enzyme activity in guinea-pigs was studied after a 32 day treatment, a period which corresponds to two oestrous cycles in this animal species. The results shown in Table 7 indicate that lynestrenol plus mestranol and norethisterone plus mestranol treatment at doses that are effective in reducing fertility in this species did not significantly modify the activity of the liver microsomal enzymes. However, the guinea-pigs were sensitive to the inducing activity of other drugs known to stimulate microsomal drug metabolism, such as phenobarbitone and eucalyptol.

Table 7 Effect of combined treatment with contraceptive drugs on liver microsomal enzyme activity in guinea-pigs

No. of animals	*Treatment (mg/kg) and route*		*No. of days*	*Enzymatic activity (nmol g^{-1} h^{+1} ± s.e.)*		
				Aminopyrine	*Aniline*	*pNO_2 Anisole*
10	Controls			302 ± 28	767 ± 37	1592 ± 132
5	Lynestrenol + mestranol	(0.3) oral (0.018)	32	374 ± 47	843 ± 35	1704 ± 67
10	Lynestrenol + mestranol	(1.25) oral (0.075)	32	311 ± 27	627 ± 23	1596 ± 88
5	Lynestrenol + mestranol	(5) oral (0.3)	32	304 ± 19	565 ± 14	1599 ± 17
5	Norethisterone + mestranol	(4) oral (0.2)	32	331 ± 18	812 ± 49	1511 ± 82
5	Phenobarbitone	(80) i.p.	2	985 ± 153*	1195 ± 76*	2836 ± 281*
5	Eucalyptol	(500) s.c.	3	769 ± 44*	847 ± 43	2188 ± 104*

The activity was measured on the supernatant of the 9000*g* fraction of the liver homogenate corresponding to 320 mg of fresh tissue. Experimental conditions as described in Table 2.
* $P < 0.01$ versus controls.

Discussion

The results described here, confirm our previous data (Jori *et al.*, 1969) and indicate that the three SCD treatments tested enhance, although to various extents, the activity of the liver microsomal enzymes in rats and mice. This effect is probably produced by the progestogenic compounds alone, because, at least for one of the combined treatments tested in two animal species, the oestrogen alone is almost devoid of activity. These results agree with those previously obtained by our laboratory (Jori *et al.*, 1969) and by other authors (Blackham & Spencer, 1969), concerning the effect of oestrogens on drug metabolism *in vivo* and *in vitro*. However a potentiating effect of the oestrogens on the activity of the progestogens cannot be excluded at least for some substrates.

We were unable to detect any decrease in the activity of the liver microsomal enzymes after combined contraceptive treatments in mice and rats. The inhibition of drug metabolism by the progestogens was generally found in experiments *in vitro* and at very high concentrations (Soyka & Long, 1972; Tüttenberg *et al.*, 1974). *In vivo* only norethynodrel has been reported to inhibit pentobarbitone metabolism (Juchau & Fouts, 1966; Jori *et al.*, 1969), after a single acute treatment. All these situations are quite different from the conditions of the *in vivo* chronic treatment used in our experiments.

The increase in liver microsomal enzyme activity after contraceptive drug treatment in mice and rats, is not associated with an increase of the microsomal proteins or of the cytochrome P 450 content, parameters generally indicative of enzyme induction. These results are not surprising because other steroids such as methyltestosterone, cortisone and spironolactone stimulate drug metabolism in female rats without increasing the cytochrome P 450 content (Hamrick, Zampaglione, Stripp & Gillette, 1973). Moreover these authors stated that the rate of drug metabolism is determined by factors which cannot be directly related to cytochrome P 450 content, cytochrome reductase activity and magnitude of spectral changes.

Our data are at variance with the findings of Freudenthal & Amerson (1974) who showed that oestrogen–progestogen combinations are not microsomal enzyme inducers. Possible explanations for these differences are the following: first their conclusions are based mainly on the lack of an increase in P 450 content and in other components of the microsomal electron transport system, after progestogenic treatment; second, they used male rats and it has been reported that other steroids increase microsomal metabolism only in female rats (Stripp, Hamrick & Zampaglione, 1970; Hamrick *et al.*, 1973); finally, in their experiments the chronic treatment was repeated for only 4 days. In our experiments contraceptive treatment was always active in mice after a few administrations whereas rats, at least for lynestrenol plus mestranol combination, needed a chronic treatment of more than 4 days to show a significant inducing effect.

However, our aim was to study the activity of liver microsomal enzymes, in experimental conditions reproducing as closely as possible, the pattern of use of the contraceptives in human therapy and consequently for periods covering more than one oestrous cycle. An objection could be raised that the modifications in microsomal enzyme activity produced by the various treatments are present only at very high doses as compared with those used in human contraceptive treatment; but it must be noted that in fact, the doses used are very low in relation to the antifertility effect exerted by such treatment in rats and mice. A significant increase in enzymatic activity appears at doses unable to protect all the animals from pregnancy.

The reactivity of the guinea-pigs to the contraceptive combined treatments is quite different from that of mice and rats. This animal species is very sensitive to the antifertility action of the contraceptive drugs, but is is unresponsive to their microsomal enzyme-inducing activity. It is difficult to explain this lack of response on the basis of a poor availability or a different kinetic behaviour of the progestogenic compounds in this animal species because they exert at these doses a high antifertility action, although a more specific distribution of the steroids in the target reproductive organs compared with the liver cannot be excluded in this species.

However, it may be more likely that this different reactivity of the guinea-pig is due to the widely recognized species differences in microsomal drug metabolism (Parke, 1968). Differences between mice and guinea-pigs in biochemical composition and enzyme distribution in microsomal sub fractions have been reported recently (Gram, Schroeder, Davis, Reagen & Guarino, 1971).

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