by a combination of both antidotes. This forms the basis of the treatment of cyanide toxicity and the mechanisms of the antidotal action are that the treatment with nitrite forms methaemoglobin and so complexes with the cyanide, and the thiosulphate accelerates the metabolic conversion of cyanide into thiocyanate. Using an experimental approach similar to that of Way, Gibbon & Sheehy (1966), the protective actions of sodium nitrite and sodium thiosulphate on the toxicity of (II) and (IIa) were studied. Results are given in Table 2. Sodium nitrite and sodium thiosulphate individually afforded some protection against the lethal effects of (IIa). However, pretreatment of mice with both sodium nitrite and sodium thiosulphate afforded increased protection against the lethal effects of (II) and (IIa). Sodium nitrite and sodium thiosulphate did not protect against lethality produced by (I). On the basis of these results we speculate that CN^- may be a metabolite of (II) and (IIa) in mice.

The potential of (IIa) to produce hepatotoxic and nephrotoxic effects was also assessed. Groups of 10 mice were dosed with vehicle (H_2O) or (IIa) at 45, 50, and 55 mg kg^{-1}, and 24 h later, blood obtained in heparinized syringes by cardiac puncture. As an index of hepatotoxicity, plasma concentrations of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were monitored. Blood urea nitrogen (BUN) was used as an indicator of nephrotoxicity. Our results show that concentrations of GOT, GPT and BUN in mice treated with (IIa) did not differ from those in control mice, indicating that (IIa) is probably not hepatotoxic or nephrotoxic.

Table 2. *Effects of sodium nitrite and sodium thiosulphate on the toxicity of 1-piperidinocyclohexanecarbonitrile and phencyclidine.*

Pretreatment[a]	Challenge[b]	No. mice dead / No. mice used
$NaNO_2$	H_2O	0/8
$Na_2S_2O_3$	H_2O	0/8
$NaNO_2 + Na_2S_2O_3$	H_2O	0/8
H_2O	(IIa)	6/8
$NaNO_2$	(IIa)	1/8
$Na_2S_2O_3$	(IIa)	3/8
$NaNO_2 + Na_2S_2O_3$	(IIa)	0/8
H_2O	(II)	5/6
$NaNO_2 + Na_2S_2O_3$	(II)	0/6
H_2O	(I)	7/10
$NaNO_2 + Na_2S_2O_3$	(I)	8/10

[a] $NaNO_2$ (20 mg kg^{-1}) and $Na_2S_2O_3$ (200 mg kg^{-1}) were administered intraperitoneally.

[b] Mice were challenged with 70 mg kg^{-1} of (IIa) or 100 mg kg^{-1} of (I) dissolved in H_2O and 180 mg kg^{-1} of (II) dissolved in corn oil, administered by oral intubation 10 min after pretreatment with $NaNO_2$ (20 mg kg^{-1}) and /or $Na_2S_2O_3$ (200 mg kg^{-1}).

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Variations of the enzyme inducing effects of contraceptive agents in different animal species

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Interactions of contraceptive steroids with the liver microsomal enzymes and in general with drug metabolism has been reported (Blackham & Spencer, 1969; Juchau & Fouts, 1966; Jori, Bianchetti & Prestini, 1969; Rümke & Noordhoek, 1969; Freudenthal & Amerson, 1974; Garg & Ahmad, 1974; Soyka & Deckert, 1974; Tüttenberg, Hüthwohl & others, 1974; Freudenthal, Amerson & others, 1974). However, various animal species and different experimental approaches and contraceptive steroid combinations were employed, with conflicting results.

We have compared the effect of some of the most frequently used combinations of steroid contraceptives on the liver microsomal enzyme activity in rat, mouse and guinea-pig.

We also report the results of previous experiments in which the antifertility activity of the contraceptive combinations (lynestrenol + mestranol; norethynodrel + mestranol and norethindrone + mestranol) was examined after an acute or chronic treatment over a range of doses to assess a dose-response relationship. The combinations were always given with fixed ratios of progestogen to oestrogen of 1:0·06; 1:0·015 and 1:0·05 respectively, to compare with ratios used in woman.

* Correspondence.

Table 1. *Effective doses of the contraceptive steroid combinations on the antifertility test.*

Treatment	Rat		Mouse		Guinea-pig
	4 Days	30 Days	4 Days	30 Days	30 Days
Lynestrenol + mestranol	2·2 0·13	2 0·12	1·8 0·11	1·65 0·099	0·82 0·049
Norethynodrel + mestranol	3 0·045	4·2 0·063	0·4 0·006	0·4 0·006	0·36 0·0054
Norethindrone + mestranol	2·2 0·11	1·8 0·09	0·85 0·042	1 0·05	0·38 0·019

Figures indicate a graphically calculated ED90 (mg kg^{-1} daily orally): dose of steroid combination which reduces the control level of implantations by 90%.
Rats and mice were killed 8 days and guinea-pigs 25 days after the last treatment.

Sexually mature female Charles River rats, CD_1 mice and PIR bright/Z guinea-pigs received orally the drug combinations daily for 4 days (corresponding to 1 oestrus cycle in rats and mice) or 30 days (corresponding to 7 oestrus cycles in rats and mice and 2 in guinea-pigs).

In the experiments on antifertility activity, the animals were caged with fertile males at the beginning of the trial (guinea-pigs, rats and mice in the 4 day treatments only). For the chronic experiments, the males were caged with the female rats and mice on the 24th day of treatment only.

Animals were killed and uterine horns examined and the number of implantations as % of controls were plotted on semilogarithmic paper against the dosages to obtain the ED90.

Animals used to examine the liver microsomal enzyme activity (caged without males) were killed 2 or 18 h after the last treatment. Enzymatic activity was measured on the 9000 *g* supernatant fraction of liver homogenates according to Kato & Takanaka (1967). Metabolites formed from the added substrates, aniline, aminopyrine and *p*-nitroanisole, were determined according to Gilbert & Goldberg (1965).

Microsomal proteins and cytochrome P 450 were measured on the microsomal pellets obtained according to the procedure of Schenkman & Cinti (1972), using the method of Lowry, Rosebrough & others (1951) and Omura & Sato (1964) respectively.

The results obtained can be summarized in the following points:

(i) The rat needed a fairly high dose of each of the three combinations to avoid implantation. The mouse needed a similar dose of lynestrenol + mestranol and norethindrone + mestranol while

Table 2. *Effect of several steroid contraceptive drugs on liver microsomal enzyme activities of several animal species.*

Species	Treatment (mg × kg oral) ×30 days	Enzymatic activity° (mμmol g^{-1} h^{-1} + s.e.)		
		N-demethylase	Aromatic hydroxylase	*O*-demethylase
	Controls			
Rat	—	230 ± 25	667 ± 68	615 ± 46
Mouse	—	392 ± 21	626 ± 22	821 ± 16
Guinea-pig	—	302 ± 28	767 ± 37	1592 ± 132
	Lynestrenol + mestranol			
Rat	(1·25 + 0·075)	379 ± 29**	829 ± 57**	803 ± 34**
Mouse	(1·25 + 0·075)	609 ± 18**	885 ± 67**	1181 ± 24**
Guinea-pig	(1·25 + 0·075)	311 ± 27	627 ± 23	1596 ± 88
Guinea-pig	(5 + 0·3)	304 ± 19	565 ± 14	1599 ±17
	Norethindrone + mestranol			
Rat	(2 + 0·1)	435 ± 36**	1233 ± 36**	794 ± 38**
Mouse	(2 + 0·1)	608 ± 39**	863 ± 22**	1092 ± 30**
Guinea-pig	(4 + 0·2)	331 ± 18	812 ± 49	1511 ± 82
	Norethynodrel + mestranol			
Rat	(2 + 0·03)	324 ± 20**	1081 ± 49**	738 ± 27**
Mouse	(2 + 0·03)	432 ± 25	672 ± 28	812 ± 29
Mouse	(4 + 0·06)	335 ± 9	511 ± 23	721 ± 42

** $P < 0{\cdot}01$ versus controls of the same animal species (Student's *t*-test).
Each figure is the average of at least 5 determinations. Animals were killed 18 h after the last administration.
(o) The enzymatic activity is determined on the liver 9000*g* supernatant fraction.
For *N*-demethylase the formation of 4-amino antipyrine from aminopyrine was measured; for aromatic hydroxylase the formation of *p*-aminophenol from aniline; for *O*-demethylase the formation of *p*-nitrophenol from *p*-nitroanisole.

small amounts of norethynodrel + mestranol were still effective. The guinea-pig was more sensitive, lower doses completely abolishing the implantations (Table 1).

(ii) The activity of the liver microsomal enzymes was not reduced or modified immediately (2 h) after the last administration either at 4 or 30 days in any species.

(iii) In contrast, in rats and mice, the activity of the liver microsomal enzymes measured 18 h after the last administration was increased by each treatment (except norethynodrel + mestranol in mice). A 4 day treatment, covering one oestrus cycle, was generally enough to stimulate these enzymatic activities. Moreover the effect appeared with doses lower than those reached to elicit a complete loss of fertility in both rat and mouse (see Table 1). The enzyme-inducing effect was related to the cessation of steroid treatment and was observed to reach a peak only 18 h after the last treatment.

(iv) The same combinations did not modify the activity of the liver microsomal enzymes in the guinea-pig although the doses used were higher than the lowest doses giving maximal antifertility activity.

(v) No effect on the liver microsomal protein content or on the cytochrome P 450 concentration was observed in any experiments.

A typical example of the results is given in Table 2. The doses used exert an antifertility activity of 50 to 100% in mice and rats. For the guinea-pigs, and also with norethynodrel for mice, the doses used were about 10 times higher than the lowest amounts capable of completely blocking fertility.

It is difficult to conclude that the combinations are typical inducers of drug metabolism since, like Freudenthal & Amerson (1974), we failed to observe an increase in liver microsomal proteins and in cytochrome P 450 concentration. However, other steroids such as methyltestosterone, cortisone and spironolactone stimulate the drug metabolism in female rats without increasing the cytochrome P 450 (Hamrick, Zampaglione & others, 1973).

It may be that factors not directly related to cytochrome P 450, such as cytochrome reductase activity or changes in substrate affinity, are responsible for the observed modifications in the rate of drug metabolism. Our results show evidence of a different reactivity to the contraceptive-induced modification of drug metabolism, in the three animal species examined. Although this fact could be ascribed to the widely recognized species differences in the drug metabolism (Parke, 1968) other explanations are possible. For instance the concentrations of contraceptives in plasma were not measured so different drug availability in the three species cannot be ruled out. The contraceptives were compared only in terms of the antifertility activity. From this point of view the doses used, although some hundred times greater than those prescribed for women, were low for rats and mice and high for guinea-pigs. Therefore there is not a close correlation between the dose of contraceptive capable of exerting antifertility activity and the dose effective in increasing liver microsomal metabolism.

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