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INTERRUPTION OF PREGNANCY BY VARIOUS STEROIDS

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Numerous earlier observations have suggested that, in the rat, various steroids can not only prevent conception, but can also interrupt pregnancy when administered after copulation. Our interest in this matter was first aroused in 1935 by observations which were summarized as follows: "In normal (not ovariectomized) rats, oestrine leads to abortion during the first part of gestation only. When given during late stages, it interferes with parturition at term."[1] Similar observations have been reported by numerous other investigators.[2-6] It was found that various follicular and other steroids may be effective, but the decisive prerequisite for the interruption of pregnancy appeared to be their "estrogenic" or folliculoid effect.

Recent investigations showed that catatoxic steroids increase the metabolic degradation of many steroid hormones, including progesterone and other luteoids. These findings raised the possibility that pregnancy might be interrupted by inducing a sex steroid deficiency in this manner.[7, 8] We felt that this interpretation could offer an explanation for the observation made during our earliest work on the possible teratogenic effect of catatoxic compounds in the rat, namely, that even steroids devoid of folliculoid properties (e.g., ethylestrenol, CS-1, and spironolactone) often interrupted pregnancy.

To clarify this problem, extensive investigations were initiated with various steroids, given alone or in combination, either during only certain stages or the entire length of gestation.

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MATERIAL AND METHODS

For all our experiments, we used ARS/Sprague-Dawley rats with an initial mean body weight of approximately 250 gm. (200–300 gm.). Each animal was kept in a separate cage and given Purina Laboratory Chow and tap water ad libitum. Insemination was determined by the identification of sperm in the vagina ("0" day of pregnancy); successful fertilization was checked by direct inspection of the uterus through a small laparotomy incision on the 8th day. After this, the development of the embryos was verified during pregnancy by palpation and eventually by the birth of viable young. However, in the event of abortion, which often takes place during the night, the mothers tend to eat the conceptus, leaving no detectable trace of it. Hence, when births failed to occur by the end of the 23rd day, the mothers were killed in order to determine the absence of fetuses or their partial intrauterine absorption. In our tables, the column "Pregnancy Interruption" gives the percentage of mothers that failed to deliver viable young because of either abortion or intrauterine absorption.

All steroids were administered in the form of aqueous suspensions (prepared in a homogenizer after addition of a few drops of Tween 80). The individual doses indicated in the tables were always given twice daily in 1 ml. of water by stomach tube. *Cholesterol* was used as a control substance having neither hormonal nor catatoxic effects. *Phenobarbital* (B.D.H.) was administered also for control purposes, as a potent non-steroidal hepatic microsomal enzyme inducer. The actual test substances were the

following: *17β-estradiol* (Roussel), *ethylestrenol* (Organon), *triamcinolone* (Lederle), *CS-1* (Catatoxic Steroid No. 1; 9α-fluoro-11β,17-dihydroxy-3-oxo-4-androstene-17α-propionic acid potassium salt; factory code number 11927, Searle), *spironolactone* (Searle), *dexamethasone* acetate (Schering), *progesterone* (Roussel), *PCN*, 3β-hydroxy-20-oxo-5-pregnene-16α-carbonitrile (Searle), *prednisolone* 21-acetate (Roussel), *dehydroepiandrosterone*, 3β-hydroxy-5-androsten-17-one (Ayerst), *oxandrolone* (Searle), and *norbolethone* (Wyeth).

RESULTS

First Experiment: Abortifacient Action of Various Steroids Administered during Different Periods of Pregnancy. In the first experiment, the test substances were given twice daily, per 100 gm. body weight, at the individual dose level of 10 mg., except for dexamethasone and phenobarbital, which, because of their toxicity, had to be administered at the dose level of 10 μg. and 3 mg., respectively. Treatment was limited to the 1st, 2nd, 3rd, or 4th 5-day period of gestation as indicated in Table 1.

TABLE 1. *Abortifacient Action of Various Steroids Given During Limited Periods of Gestation*

Steroid	Period of gestation	No. of rats	Pregnancy interruption
(mg./100 gm./dose)	*(days)*		(%)
Cholesterol (10)	2–6	10	0
	4–8	8	0
	9–13	10	0
	15–19	7	0
Phenobarbital (3)	2–6	9	11
	4–8	9	22
	9–13	11	0
	15–19	8	0
Estradiol (10)	2–6	11	100
	4–8	10	100
	9–13	10	100
	15–19	9	100
Ethylestrenol (10)	2–6	12	100
	4–8	10	40
	9–13	9	22
	15–19	7	100 (stillborn)
Triamcinolone (10)	2–6	11	73
	4–8	10	100
	9–13	11	100 (stillborn)
	15–19	9	100 (stillborn)
CS-1 (10)	2–6	12	67
	4–8	10	80
	9–13	9	33
	15–19	9	0
Spironolactone (10)	2–6	10	0
	4–8	10	20
	9–13	11	91
	15–19	9	11
Dexamethasone (0.01)	2–6	9	0
	4–8	8	10
	9–13	6	0
	15–19	8	11
Progesterone (10)	2–6	11	0
	4–8	10	0
	9–13	10	0
	15–19	9	0
PCN (10)	2–6	9	0
	4–8	8	0
	9–13	9	0
	15–19	10	0

As expected, cholesterol failed to interrupt pregnancy. However, surprisingly, phenobarbital, one of the most potent nonsteroidal microsomal enzyme inducers, likewise proved to be virtually ineffective in this respect: during the first quarter of gestation, only one female aborted and during the second quarter, two females aborted. Such a small percentage of miscarriages may have been due to chance.

On the other hand, under similar conditions, pregnancy interruption was invariably achieved by estradiol when given at any stage of gestation. Ethylestrenol was equally efficacious during the first stage, triamcinolone during the second stage, and spironolactone, almost equally, during the third stage. CS-1 terminated pregnancy in a significant number of cases during the first, and even more constantly during the second, stage of gestation, but gradually it became ineffective as pregnancy approached its end. Under our conditions, dexamethasone was virtually, and progesterone and PCN totally, devoid of abortifacient potency at any stage of gestation.

TABLE 2. *Abortifacient Action of Various Steroids Administered from the 2nd to the 19th Day of Pregnancy*

Steroid	No. of rats	Pregnancy interruption
(*mg./100 gm./dose*)		(%)
Cholesterol (10)	9	0
Phenobarbital (3)	8	0
Prednisolone (10)	11	100
Dehydroepiandrosterone (10)	12	100
Oxandrolone (10)	10	100
Norbolethone (10)	10	80
Ethylestrenol (10)	10	100
Triamcinolone (0.5)	9	45
CS-1 (10)	11	100
Dexamethasone (0.01)	7*	100
PCN (10)	10	0

* Three rats of this group died between the 16th and the 19th day of the experiment.

Second Experiment: Abortifacient Action of Various Steroids Administered throughout Pregnancy. In order to further explore the possible abortifacient action of steroids, a second experiment was performed in which these compounds were administered from the day after conception to the 19th day, that is, virtually throughout the entire period of pregnancy.

As shown by Table 2, even under these conditions, phenobarbital, cholesterol, and PCN failed to produce abortion, whereas prednisolone, dehydroepiandrosterone, oxandrolone, ethylestrenol, CS-1, and dexamethasone produced 100%, norbolethone 80%, and triamcinolone (at this small dose) 45%, abortions and/or absorptions.

Third Experiment: Abortifacient Action of Various Steroids Given Singly or in Combination at Comparatively Small Dose Levels between the 2nd and 6th Day of the Gestation. As shown in Table 3, at the dose of 1 mg., estradiol, whether administered alone or in combination with ethylestrenol, was sufficient to terminate pregnancy in all the animals. When given singly, ethylestrenol rarely produced abortion at the 5-mg. dose level, and its efficacy in this respect was only slightly enhanced by combined treatment with CS-1. By itself, the latter proved to be ineffective under these conditions. At the 1-mg. dose level, triamcinolone caused no interruption of pregnancy and also failed to significantly enhance the abortifacient action of ethylestrenol.

Fourth Experiment: Effect of Gestation upon the Catatoxic Action of PCN against Digitoxin, Indomethacin, and Progesterone. It was unexpected that, during the first quarter of gestation, some of the most potent catatoxic steroids, such as PCN or spironolactone, failed to interrupt pregnancy. It is well known that, in the rat, the maintenance of early pregnancy is dependent upon the availability of progesterone, and earlier experiments[7, 8] clearly showed that even enormous (anesthesia-producing) amounts of progesterone are readily inactivated by these and many other catatoxic steroids. The question arose therefore whether, during pregnancy, there exists some mechanism which interferes with the induction of hepatic microsomal drug- and steroid-metabolizing enzymes by otherwise potent inducers, or whether endogenous corpus luteum hormone, as secreted during gestation, is more resistant to this type of detoxication than exogenous progesterone.

In order to clarify this point, a fourth experimental series was performed in which the responsiveness of three substrates (pro-

TABLE 3. *Abortifacient Action of Various Steroids Given Singly or in Combinations at Comparatively Small Dose Levels between the 2nd and 6th Day of Gestation*

Steroid	No. of rats	Pregnancy termination
(*mg./100 gm./dose*)		(%)
Cholesterol (10)	5	0
Ethylestrenol (5)	7	28
CS-1 (5)	7	0
Estradiol (1)	6	100
Triamcinolone (1)	5	0
Ethylestrenol (5) + CS-1 (5)	6	66
Ethylestrenol (5) + Estradiol (1)	6	100
Ethylestrenol (5) + Triamcinolone (1)	6	50

TABLE 4. *Effect of Gestation upon the Catatoxic Action of PCN against Digitoxin, Indomethacin, and Progesterone*

Pretreatment	Digitoxin				Indomethacin				Progesterone: (sleeping time at dose*)			
	Convulsions (positive/total)		Mortality (dead/total)		Intestinal ulcers (positive/total)		Mortality (dead/total)		10 mg.		15 mg.	
	Non-pregnant	Pregnant (8th day)	Non-pregnant	Pregnant (10th day)	Non-pregnant	Pregnant (10th day)	Non-pregnant	Pregnant (10th day)	Non-pregnant	Pregnant (5th day)	Non-pregnant	Pregnant (7th day)
										(*min.*)		
None	7/7	11/11	7/7	11/11	5/5	12/12	2/5	11/12	0 (5)	22 (10)	202 (8)	199 (10)
PCN			0/7	0/11	0/5	0/10	0/5	0/10	0 (5)	0 (11)	0 (5)	0 (11)

* Numbers in parentheses indicates number of rats in experiment.

gesterone, digitoxin, and indomethacin) to detoxication by PCN was compared in nonpregnant and pregnant rats. Digitoxin (2 mg. in 1 ml. of water/100 gm. body weight perorally) was given on the 5th and 6th days. Indomethacin (1 mg. in 0.2 ml. of water/100 gm. body weight subcutaneously was injected daily between the 5th and the 9th day of gestation. Progesterone was administered twice: 10 mg. on the 5th, and 15 mg. on the 7th day, in 1 ml. of corn oil/100 gm. body weight intraperitoneally. It is well known that, ordinarily, under these conditions, digitoxin produces convulsions, indomethacin, multiple perforating intestinal ulcers (both associated with high mortality), and progesterone, anesthesia. As shown by earlier observations in nonpregnant animals, all these signs of intoxication can be prevented by pretreatment with PCN. Hence, in the present experiment, one series of rats received these compounds without pretreatment, whereas the second series was pretreated with PCN (10 mg. in 1 ml. of water/100 gm. body weight, twice daily), beginning on the 2nd day of gestation and continuing throughout the period of observation.

As shown by Table 4, in itself, the state of gestation did not significantly alter resistance to digitoxin, indomethacin, or progesterone overdosage. Ten milligrams of progesterone intraperitoneally, which usually produces severe anesthesia in young 100-gm. female rats, caused only short or no sleep in the older females of this series; however, this comparative insensitivity could not be due to the state of gestation since it was also evident in the nonpregnant controls of the same age. On the other hand, even large doses of progesterone, which normally cause loss of righting reflex for more than 3 hr., were totally inactivated by PCN, both in pregnant and in nonpregnant animals. The same was true of digitoxin and indomethacin. Evidently, pregnancy does not interfere with the ability of PCN to detoxify either progesterone or the other two toxicants tested. Since, in the rat, early pregnancy cannot continue in the absence of abundant amounts of corpus luteum hormone, it must be assumed that, despite PCN treatment, sufficient quantities of progesterone remained available to the embryos in this experimental series.

DISCUSSION

During pregnancy, the normal production of folliculoid estrane derivatives is much lower in rats than in women. Hence, it is not unexpected that women can better tolerate additional amounts of exogenous estradiol than rats, without danger to the embryo. This fact had been mentioned by many earlier investigators and is confirmed by the present observations.

It is more interesting that even compounds devoid of folliculoid potency (e.g.,

ethylestrenol, triamcinolone, CS-1, and spironolactone) can interrupt gestation in the rat, when administered during certain periods of pregnancy. This abortifacient action is also shared by prednisolone, dehydroepiandrosterone, oxandrolone, and norbolethone, at least when administered during the entire length of gestation.

It remains very doubtful to what extent these observations on rats are reproducible in women. In any event, it is noteworthy that various catatoxic steroids are singularly ineffective in terminating pregnancy, even when given at dose levels amply sufficient to accelerate the degradation of exogenous progesterone. This is true even when the latter is administered acutely at the enormous dose levels necessary to produce anesthesia. Although during gestation, a very high blood level of progesterone must be maintained to ensure the viability of the placenta and fetus, this is still far below that required to induce sleep. Evidently, here, as in so many other instances enumerated elsewhere,[7, 8] catatoxic steroids are much more efficacious in accelerating the biotransformation of steroid hormones at excessive blood levels than at near physiologic concentrations.

Yet, steroid detoxication by enzyme inducers does not appear to be impossible at near physiologic concentrations. It has been shown repeatedly that the uterotropic effect of small doses of estradiol is effectively inhibited in the rat by pretreatment with phenobarbital;[9-13] indeed, it has even been said to cause abortion in rats.[14] This claim appears to be in conflict with our own observations; however, it is based on experiments in which very large doses (50 mg./kg.) of phenobarbital were administered intraperitoneally every day throughout gestation. Even under these conditions, embryonic mortality amounted only to 37.6% after phenobarbital, in comparison with 28.4% in the control rats.

We do not know by what means endogenous progesterone is so efficiently protected against the catatoxic action of steroids. However, in any event, this protection is by no means complete, and further experiments will be required to elucidate its mechanism and to show whether it could be overcome by appropriate pharmacologic measures.

SUMMARY

Some catatoxic steroids (ethylestrenol, CS-1, spironolactone, prednisolone, dehydroepiandrosterone, oxandrolone, and norbolethone) share with folliculoids, such as estradiol, the ability to interrupt pregnancy in the rat.

Other highly potent hepatic microsomal enzyme inducers, such as pregnenolone-16α-carbonitrile (PCN), and phenobarbital, appear to be virtually devoid of this abortifacient effect.

Earlier work had shown that all catatoxic steroids greatly accelerate the metabolic degradation of exogenous progesterone when the latter is administered at very high (anesthetic) dose levels; hence, there appears to exist some mechanism through which the endogenous progesterone, required to maintain pregnancy, is protected against this type of catatoxic effect.

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REFERENCES

1. SELYE, H. Hormonal interrelations during pregnancy. (Proc. 15th int. physiol. Congr., Leningrad, Moscow, 1935). *Sechenov J Physiol USSR 21:* 205, 1938.
2. CHAMBON, Y. Précisions quantitatives sur l'avortement oestrogénique pendant et après l'ovoimplantation chez la lapine. *CR Soc Biol (Paris) 149:* 2164, 1955.
3. GREENWALD, G. S. The anti-fertility effects in pregnant rats of a single injection of estradiol cyclopentylpropionate. *Endocrinology 69:*1068, 1961.
4. KELLER, T., AND SKOWRON, S. W sprawie ronnego

dzialania follikuliny. (A propos de l'action abortive de la folliculine.) *Ginek Pol 13:*1, 1934.
5. MAROIS, M. Sur l'action nocive exercée sur la gestation par certains stéroïdes. *CR Soc Biol (Paris) 154:*1361, 1960.
6. PINCUS, G. *The Control of Fertility*. Acad. Press, New York, 1965, p. 360.
7. SELYE, H. Hormones and resistance. *J Pharm Sci 60:*1, 1971.
8. SELYE, H. *Hormones and Resistance*. Springer-Verlag, New York, 1971, p. 1200.
9. CONNEY, A. H. Stimulatory effect of drugs on drug metabolism (abstr.). *Pharmacologist 9:*77, 1967.
10. FAHIM, M. S., KING, T. M., VENSON, V., NORWICH, C., AND BOLT, D. J. Uterotropic action of estrogens in phenobarbital-treated mice. *Fertil Steril 20:*344, 1969.
11. LEVIN, W., AND CONNEY, A. H. Effect of phenobarbital on the uterine response to estradiol-17β (abstr.). *Fed Proc 25:*251, 1966.
12. LEVIN, W., WELCH, R. M., AND CONNEY, A. H. Effect of chronic phenobarbital treatment on the liver microsomal metabolism and uterotropic action of 17β-estradiol. *Endocrinology 80:*135, 1967.
13. SINGHAL, R. L., VALADARES, J. R. E., AND LING, G. M. Influence of chronic phenobarbitone treatment on uterine phosphofructokinase induction. *J Pharm Pharmacol 19:*545, 1967.
14. FAHIM, M. S., HALL, D. G., JONES, T. M., FAHIM, Z., AND WHITT, F. D. Drug-steroid interaction in the pregnant rat, fetus, and neonate. *Amer J Obstet Gynec 107:*1250, 1970.