mammogenesis and luteal function during pseudopregnancy.

1. Schwartz, N. B. and Rothchild, I., Proc. Soc. Exptl. Biol. Med. 116, 107 (1964).
2. Wrenn, T. R., Bitman, J., DeLauder, W. R., and Mench, M. L., J. Dairy Sci. 49, 183 (1966).
3. Herlyn, U., Geller, H.-F., Berswordt-Wallrabe, I. V., and Berswordt-Wallrabe, R. V., Acta Endocrinol. 48, 220 (1965).
4. De Feo, V. J., Endocrinology 79, 440 (1966).
5. Reece, R. P. and Turner, C. W., Missouri, Univ. Agr. Expt. Sta. Res. Bul. 226, 8 (1937).
6. Bliss, C. I., "The Statistics of Bioassay." Academic Press, New York (1952).
7. Sinha, Y. N. and Tucker, H. A., Proc. Soc. Exptl. Biol. Med. 128, 84 (1968).
8. Taleisnik, S., Caligaris, L., and Astrada, J. J., Endocrinology 79, 49 (1966).
9. Desjardins, C., Kirton, K. T., and Hafs, H. D., Proc. Soc. Exptl. Biol. Med. 126, 23 (1967).
10. Sawyer, C. H. and Markee, J. E., Endocrinology 65, 614 (1959).
11. Anderson, R. R. and Turner, C. W., Proc. Soc. Exptl. Biol. Med. 128, 210 (1968).

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Effect of an Adrenal 17α-Hydroxylase Inhibitor on Gonad Function (33676)

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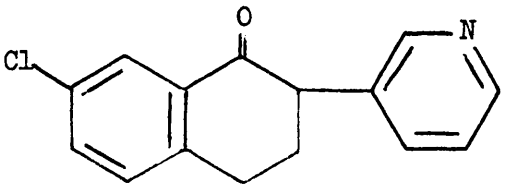
Since the original work with amphenone (1) and related compounds such as metyrapone, a large number of substances were shown to modify the pattern of steroidogenesis in the adrenal [Refs. (2, 3)]. One of these, designated Su-10603, synthesized by Bencze and Barsky (4) had considerable activity as an adrenal 17α-hydroxylase inhibitor both *in vivo* and *in vitro* (5–7).



7-chloro-3,4-dihydro-2-(3-pyridyl)-1(2*H*)-napthalenone (Su-10603)

In the dog it decreased the secretion of cortisol and 11-desoxycortisol, resulting in a compensatory increase in the secretion of corticosterone and 11-desoxycorticosterone.

This raised the question as to its possible effect on the secretion of gonadal hormones. It and related compounds have, indeed, been found to inhibit androgen synthesis *in vitro* in various systems and circumstances (8–15). The only report of gonadal effects in intact animals, however, is one in which an inhibition in male rats of the acute response to chorionic gonadotropin was found (6). The present report extends that work and includes the results of long-term treatment with Su-10603 in both male and female rats.

Methods. Two types of tests were done. In one, immature male and female rats (50–60 g initially) were treated daily for 30 days with varying doses of Su-10603 s.c. The drug was dissolved in polyethelene glycol at a concentration of 12.5–200 mg/ml. At the termination of the experiment, organ weights were taken and some tissues studied histologically.

In the second type of experiment rats like those used above were treated daily for 4 days simultaneously with Su-10603 and 2 units/rat of chorionic gonadotropin (APL-Ayerst). Autopsy was performed and organ weights obtained on the fifth day.

Corticosterone secretion rates were measured using whole blood from the left adrenal vein. Methods were based on those of Knigge (15) as described for the hamster.

Results. Non-effect on corticosterone secretion rate. If, as evidence cited above indicates, Su-10603 is a more or less specific inhibitor of 17α-hydroxylation, it might be expected not to alter the corticosterone secretion rate in rats. That was the case when 15

daily doses (100 mg/kg) were given to males, s.c., the last injection being made 1 hr before determinations:

Treatment	Corticosterone secretion (μg/min $\pm$ SE)	No. of rats
Vehicle controls	0.439 $\pm$ 0.051	12
Su-10603 (100 mg/kg/day; $\times$15)	0.448 $\pm$ 0.062	12

Furthermore, as noted below, there were no morphological signs that the adrenals were affected by the drug.

Thirty-day treatment. As seen in Table I, sex accessory weights of males and uterine weights of females were significantly inhibited by treatment with Su-10603 in doses of 100–400 mg/kg/day. The responses were not sharply dose-related and in no case did the inhibition equal the effects of castration, as judged by a castrate control group (not shown in tables). Absolute gonad weights were unaffected and relative weights changed only by the dose that reduced body growth. No alterations in gonad histology were seen.

Adrenal weight and histology were not appreciably affected. The lack of adrenal hypertrophy, such as is seen with other compounds of this general pharmacological type, e.g., amphenone or metyrapone, suggests that Su-10603 caused little adrenal inhibition of the type seen in dogs. Probably this is because the rat adrenal normally synthesizes little if any 17-hydroxylated steroid under usual circumstances (16).

Weights of the kidney, pituitary, and thyroid were unaffected by 30-day treatment with Su-10603 as was thyroid histology.

In 7 adrenalectomized animals given 100 mg/kg/day for a month, Su-10603 manifested its characteristic depression of seminal vesicle growth (—26%). The animals were maintained during treatment with 1% NaCl to drink and 0.01 mg of dexamethasone/rat three times per week.

Response to chorionic gonadotropin, (Table II). It had been reported earlier in male rats (6) that the response to chorionic gonadotropin was inhibited by 100 mg/kg/day of Su-10603 and the detailed figures on which that conclusion was based, together with the

TABLE I. Effect of Su-10603 in Immature Rats (30-day treatment).

Treatment and dose (mg/kg/day)	No. of rats and sex	Wt. gain (%)	Organ wt. (mg/100 g)				
			Adrenal	Testis	Seminal vesicle	Ventral prostate	Levator ani
Controls (vehicle)	8 ♂	284	14.2	1170	55.2	75.0	56.4
Su-10603 (400)	8 ♂	228	12.4	1290	24.8	40.0	32.1
Diff. between means (p)		.001	NS[a]	.05	<.001	<.001	<.001
Controls (vehicle)	8 ♂	218	15.3	1270	54.7	74.3	57.1
Su-10603 (200)	8 ♂	239	14.9	1270	35.1	49.3	42.5
Diff. between means (p)		NS[a]	NS	—	.01	.01	.01
Controls (vehicle)	14 ♂	264	16.1	1260	63.6	81.2	47.0
Su-10603 (100)	14 ♂	260	14.7	1210	37.4	56.9	35.9
Diff. between means (p)		NS	NS	NS	<.001	<.001	.01
				Ovary	Uterus		
Controls (vehicle)	8 ♀	171	23.8	31.9	182		
Su-10603 (200)	8 ♀	184	25.9	33.2	132		
Diff. between means (p)		NS	NS	NS	.05		
Controls (vehicle)	8 ♀	209	26.3	—	214		
Su-10603 (100)	8 ♀	198	24.5	—	130		
Diff. between means (p)		NS	NS	—	.01		

[a] NS = not significant.

TABLE II. Su-10603: Effect on Response to Chorionic Gonadotropin 5-Day Test in Males.

Treatment	No. of rats	Wt. gain (%)	Organ wt. (mg/100 g)				
			Adrenal	Testis	Seminal vesicle	Ventral prostate	Levator ani
1. Controls (vehicle)	18	34	22.6	653	15.0	51.6	23.1
2. CG (2 U/rat/day)	18	34	21.4	615	43.1	101.2	32.9
3. Su-10603 (100 mg/kg)	18	27	23.0	561	14.2	51.0	22.1
4. Su-10603 + CG	18	26	21.6	615	25.0	91.8	24.6
Diff. between means (p)							
2 vs 4			NS[a]	NS	.01	NS	<.001
1. Controls (vehicle)	12	38	20.6	770	18.7	63.8	25.5
2. CG (2 U/rat/day)	12	35	20.3	740	42.5	101.7	41.0
3. Su-10603 (50 mg/kg)	12	38	21.7	710	15.4	58.7	28.1
4. Su-10603 (50 mg/kg) + CG	12	35	19.3	685	35.3	89.4	30.1
5. Su-10603 (25 mg/kg)	12	37	22.2	705	19.3	56.7	28.6
6. Su-10603 (25 mg/kg) + CG	12	39	21.4	720	46.1	94.4	36.0
Diff. between means (p)							
2 vs 4			NS	NS	NS	NS	<.001
2 vs 6			NS	NS	NS	NS	.01

[a] NS = not significant.

effects of smaller doses, are shown here. The minimal effective dose is near 100 mg/kg; 50 mg/kg caused less inhibition of sex accessories and 25 mg/kg was without effect except to reduce the growth response of the levatores ani. Interestingly enough this latter organ was the most sensitive indicator of an inhibitory effect of Su-10603 on organ weight responses to chorionic gonadotropin. Ventral prostates showed little response.

Curiously the response of females to chorionic gonadotropin, unlike males, was not affected by 100 mg/kg/day of Su-10603 although they did respond by uterine inhibition to 30-day treatment (Table I).

Effect on estrus, fertility, parturition. Fourteen mature female rats were given either 30 or 200 mg/kg/day of Su-10603 for 2 weeks and vaginal smears observed daily. Neither dose affected estrous cycles. An average of 2.8 estrous smears were recorded both in vehicle controls and in animals receiving drug.

At the end of this period, with medication of the females continuing, two normal males were added to each cage with a treated female for 2 additional weeks. Five of 6 animals receiving 30 mg/kg/day became pregnant and delivered normally (vs 6 of 6 controls). In the group receiving 200 mg/kg/day pregnancy, as judged by eventual delivery, occurred in 5 of 8 cases (vs 7 of 8 controls). Some reproductive dysfunction was indicated, however, by the fact that parturition in two of the treated animals on the high dose was preceded by vaginal bleeding and the litters of three animals were found dead.

Discussion. Su-10603 can be added to a growing list of compounds of various chemical types that interfere with gonad function *in vivo* by one or another means. It has been shown that Su-10603 itself (13, 14), as well as closely related compounds, inhibits testosterone synthesis *in vitro* and this fact plus its known analogous actions on adrenal steroidogenesis both *in vitro* and *in vivo* suggests strongly that it acted in our male animals by an inhibition of androgen synthesis. Its uterine-inhibiting action in females may well be due to a similar inhibition of estrogen synthesis but confidence in that hypothesis is less certain than in the male: there is no direct *in vitro* evidence that Su-10603 affects ovarian steroidogenesis and an additional troubling fact is that it did not inhibit the response to chorionic gonadotropin in females

as it did in males. This is without obvious explanation at the moment. In contrast, however, aminoglutethimide, an inhibitor of steroidogenesis prior to the formation of Δ5-pregnenolone in the adrenal, selectively interferes with ovarian function without evidence of effect on the testis (unpublished observations). The clear disturbance of pregnancy induced by Su-10603 awaits analysis and may or may not be related to interference with the endocrine functions of the placenta.

The type of action displayed by Su-10603 in males could be of potential clinical utility. The required dose, however, would probably be large. Also it is an open question whether the lack of marked effects of Su-10603 on the rat's adrenal as shown here, and the attendant lack of toxicity, would obtain in other species such as the dog and man which depend on 17-hydroxylated adrenal steroids. The rat adrenal may escape important inhibitory response to the drug only because of its normally different pattern of steroid output.

Summary. 7-Chloro-3,4-dihydro-2-(3-pyridyl)-1(2*H*)-napthalenone (Su-10603), a compound known to inhibit particularly 17α-hydroxylation in the adrenal of the dog, was studied for its effects on gonad and adrenal function in rats. Administration to immature animals for 30 days (100–400 mg/kg/day) inhibited growth of seminal vesicles, ventral prostates, levatores ani and uteri without other obvious effects. It was similarly active in adrenalectomized rats. These results are consistent with previous demonstrations that the drug interferes with testosterone synthesis *in vitro*. The Su-10603 also inhibited the response of immature males but not females to chorionic gonadotropin as judged by weights of sex accessories. It interfered with the normal processes of pregnancy by means as yet undetermined. Unlike the situation in the dog, it was without effect on adrenal morphology or the corticosterone secretion rate in rats.

The statistical work recorded here was done by Mrs. Hanna Sylwestrowicz and her associates, Biostatistics Section of this laboratory. The corticosterone determinations were made by Mrs. Jean Dolan and Dr. Innes Cargill, Biochemistry Division of this laboratory.

1. Hertz, R., Tullner, W. W., Schricker, J. A., Dhyse, F. G., and Hallman, L. F., Recent Progr. Hormone Res. **11**, 119 (1955).

2. Gaunt, R., Chart, J. J., and Renzi, A. A., Ergeb. Physiol. **56**, 114 (1965).

3. Gaunt, R., Steinetz, B. G., and Chart, J. J., Clin. Pharmacol Therap. **9**, 657 (1968).

4. Bencze, W. L. and Barsky, L. I., J. Med. Pharm. Chem. **5**, 1298 (1962).

5. Chart, J. J., Sheppard, H., Mowles, T., and Howie, N., Endocrinology **71**, 479 (1962).

6. Chart, J. J. and Sheppard, H., *in* "Hormonal Steroids" (L. Martini and A. Pecile, eds.) Vol. 1, p. 399. Academic Press, New York (1964).

7. Kahnt, F. W. and Neher, R., Experientia **18**, 499 (1962).

8. Hall, P. F. and Eik-Nes, K. B., Federation Proc. **21**, 197 (1962).

9. Ichii, S., Forchielli, E., Cassidy, C. E., Rosoff, C. B., and Dorfman, R. I., Biochem. Biophys. Res. Comm. **9**, 344 (1962).

10. Hall, P. F., Eik-Nes, K. G., and Samuels, L. T., Endocrinology **73**, 547 (1963).

11. Hall, P. F. and Eik-Nes, K. B., Biochim. Biophys. Acta **86**, 604 (1964).

12. Grosso, L. L., Am. Zool. **4**, 414 (1964).

13. Neher, R. and Kahnt, F. W., Experientia **21**, 1 (1965).

14. Shikita, M., Ogiso, T., and Tamaoki, B., Biochim Biophys. Acta **105**, 516 (1965).

15. Colla, J. C., Liberti, J. P., and Ungar, F., Steroids **8**, 25 (1966).

16. Knigge, K. M., Anat. Record **141**, 145 (1961).

17. Young, R. B. and Sweat, M. L., Arch. Biochem. Biophys. **121**, 576 (1967).

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