

Effect of Norethindrone on Submandibular Glands and Dental Caries in Female Rats* (33871)

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It has been reported that hydrocortisone (1), estrogens (2-4) and contraceptive steroids (5) administered chronically in relatively large doses inhibited the growth and development of rat submandibular glands. In contrast, androgens (6, 7), progesterone, and medroxyprogesterone acetate (unpublished) were found to stimulate the growth and development of these glands. It appeared, in general, that, among the steroids, those exerting adrenocorticoid or estrogenic activities inhibited while those exerting androgenic or gestogenic activities stimulated the growth and development of the submandibular glands. Since norethindrone (17- α -ethynyl-17- β -hydroxy-4-estren-3-one), one of the ingredients of some oral contraceptives, possesses gestogenic, androgenic, and anabolic activities (8), it stimulated our interest to determine the effect of prolonged administration of this agent on the growth and development of submandibular glands in immature female rats. Since it has been reported that some contraceptive steroids (5) caused an increase in the incidence of dental caries in rats, the cariogenic activity of norethindrone also was studied in the present work.

Materials and Methods. Weanling female Sprague-Dawley strain rats were divided randomly into desired groups in each of the following experiments. They were fed with cariogenic diet and supplied with distilled water *ad libitum*. The experimental procedures were the same as previously described (1,3).

Experiment 1. The duration of the experiment was 9 weeks and the test animals were given, beginning on day 23, daily (except Sundays) subcutaneous injections of 30 or 60 μ g of norethindrone¹/100 g of body weight. The daily required doses of norethindrone were dissolved in 0.1 ml of steroid diluent. Controls were treated similarly with steroid vehicle.

Experiment 2. This experiment was conducted later under identical procedures as expt. 1, but the duration of the experiment was prolonged to 14 weeks. Rats in the test groups were given 30, 60, or 90 μ g of norethindrone. For comparison, rats in a fourth group were treated similarly with 300 μ g of estriol,² since this dose level of estriol was found by our experience to be cariogenic in female rats. Controls were treated similarly with steroid vehicle alone.

Results. Experiment 1. Table I shows that the gain of body weight of both norethindrone-treated groups of rats increased significantly as compared to the controls. Norethindrone treatments resulted in a slight but not significant increase in the number and severity of carious lesions in comparison with those of the controls. Compared with the controls, the wet weights of submandibular and parotid glands of both treated groups of rats increased slightly but not significantly. The relative weights of submandibular and parotid glands (gland wt to body wt ratio) of both treated groups of rats were essentially the same as the controls. Both mean absolute and relative weights of ovaries as well as uteri decreased proportionately with increase in daily doses of norethindrone.

Administration of 30 or 60 μ g of norethin-

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¹ Ortho Research Foundation, Raritan, New Jersey.

² Estriol, Nutritional Biochemical, Inc., Cleveland, Ohio.

TABLE I. Effect of Norethindrone on Dental Caries, Salivary Glands, and Reproductive Organs in Female Rats.^f

	Vehicle		Norethindrone	
	(μ g): No. of rats:	(13)	30 (13)	60 (12)
Body wt (g)				
Final		211.5 $\pm$ 4.2 ^g	224.5 $\pm$ 3.7 ^{ah}	228.1 $\pm$ 4.1 ^o
Gain		168.9 $\pm$ 3.6	184.3 $\pm$ 3.0 ^d	186.8 $\pm$ 3.6 ^d
Dental caries				
No. of lesions/rat		3.2 $\pm$ 0.67	4.4 $\pm$ 0.66 NS	4.5 $\pm$ 0.58 NS
Severity/lesion/rat		1.026 $\pm$ 0.024	1.084 $\pm$ 0.057 NS	1.076 $\pm$ 0.077 NS
Submandibular gland wt				
(mg)		408.4 $\pm$ 12.4	425.9 $\pm$ 9.1 NS	420.8 $\pm$ 7.7 NS
(mg/100 g of body wt)		194.0 $\pm$ 5.0	190.0 $\pm$ 3.7 NS	184.9 $\pm$ 3.9 NS
Parotid gland wt				
(mg)		310.3 $\pm$ 9.4	333.5 $\pm$ 10.9 NS	319.4 $\pm$ 10.4 NS
(mg/100 g of body wt)		147.5 $\pm$ 4.1	148.6 $\pm$ 4.2 NS	140.1 $\pm$ 4.6 NS
Ovarian wt				
(mg)		71.3 $\pm$ 2.90	69.9 $\pm$ 2.80 NS	49.1 $\pm$ 3.15 ^o
(mg/100 g of body wt)		31.3 $\pm$ 2.80	31.1 $\pm$ 1.20 NS	21.5 $\pm$ 1.33 ^d
Uterine wt				
(mg)		306.2 $\pm$ 24.5	283.2 $\pm$ 17.9 NS	217.8 $\pm$ 7.9 ^d
(mg/100 g of body wt)		145.2 $\pm$ 11.3	126.5 $\pm$ 8.0 NS	95.8 $\pm$ 3.9 ^o

^f Duration of experiment = 9 weeks.^g Mean $\pm$ SE.^h *p* values compared to controls: ^a *p* < 0.05; ^o *p* < 0.01; ^d *p* < 0.005; ^e *p* < 0.001; NS = not significant.

drone did not cause significant changes in wet weights of adrenal (56.4 ± 1.4 and 55.2 ± 2.3 mg, respectively) and thyroid (9.1 ± 0.32 and 9.0 ± 0.28 mg, respectively) glands as compared to the controls (adrenal, 59.3 ± 1.3 mg; and thyroid, 9.0 ± 0.32 mg).

Experiment 2. Table II shows that administration of norethindrone in daily doses of 30 or 60 μ g for 14 weeks did not have a significant influence on the gain of body weight. However, administration of daily doses of 90 μ g of norethindrone or 300 μ g of estriol resulted in a significant decrease in body weight gain as compared to the controls. The mean number of carious lesions of all four treated groups of rats increased significantly as compared to the controls; while a significant increase in mean severity of carious lesions was only shown in rats treated with 300 μ g of estriol.

Both mean absolute and relative wet weights of submandibular and parotid glands

of all three norethindrone-treated groups of rats were essentially the same as the controls (except a significant increase in mean relative weight of submandibular gland in rats treated with 90 μ g of norethindrone). Estriol caused a decrease in absolute but an increase in relative weight of submandibular and parotid glands.

Histologically, the relative number of granular tubules (number of tubules per square mm of tissue) of 30 μ g norethindrone-treated rats increased significantly but the diameter of these tubules and the sizes of acinar cells, which were inversely related to acinar cell counts, of submandibular glands were essentially the same as the controls. However, at higher daily dose levels (60 or 90 μ g) norethindrone caused a significant increase in relative number and diameter of granular tubules as well as a significant increase in sizes of acinar cells of submandibular glands. In contrast, estriol treatment resulted in a sig-

TABLE II. Effect of Norethindrone on Dental Caries and Salivary Glands in Female Rats.^c

	Vehicle			Norethindrone			Estriol	
	(μ g): No. of rats:	(14)	30 (16)	60 (16)	90 (14)	300 (18)		
Body wt (g)								
Final	250.4 $\pm$ 3.9 ^e		254.5 $\pm$ 4.7	NS ^a	251.9 $\pm$ 4.2	NS	237.9 $\pm$ 3.4 ^b	140.3 $\pm$ 2.3 ^c
Gain	204.0 $\pm$ 3.7		208.4 $\pm$ 4.6	NS	205.9 $\pm$ 4.4	NS	190.6 $\pm$ 3.5 ^b	92.0 $\pm$ 2.3 ^c
Dental caries								
No. of lesions/rat	5.8 $\pm$ 0.37		7.5 $\pm$ 0.44 ^c		6.9 $\pm$ 0.39 ^a		7.4 $\pm$ 0.43 ^c	7.8 $\pm$ 0.50 ^d
Severity/lesion/rat	1.38 $\pm$ 0.093		1.27 $\pm$ 0.066	NS	1.28 $\pm$ 0.102	NS	1.33 $\pm$ 0.110	2.19 $\pm$ 0.142 ^e
Submandibular gland								
Wt (mg)	471.0 $\pm$ 8.9		470.8 $\pm$ 8.1	NS	490.4 $\pm$ 9.6	NS	482.7 $\pm$ 15.0	312.4 $\pm$ 5.8 ^c
(mg/100 g of body wt)	188.5 $\pm$ 3.8		185.6 $\pm$ 3.9	NS	195.2 $\pm$ 4.2	NS	202.9 $\pm$ 4.6 ^b	224.3 $\pm$ 4.6 ^c
Granular tubules (no./mm ²)	79.6 $\pm$ 0.65		83.6 $\pm$ 0.77 ^c		83.8 $\pm$ 0.80 ^c		83.6 $\pm$ 0.64 ^c	60.8 $\pm$ 1.04 ^c
Diameter (μ)	42.8 $\pm$ 0.15		42.9 $\pm$ 0.17	NS	43.4 $\pm$ 0.16 ^b		43.3 $\pm$ 0.11 ^c	37.4 $\pm$ 0.10 ^c
Acinar cell counts ^d	17.9 $\pm$ 0.21		17.7 $\pm$ 0.13	NS	15.3 $\pm$ 0.32 ^c		15.9 $\pm$ 0.23 ^c	17.8 $\pm$ 0.23 NS
Parotid wt								
(mg)	378.9 $\pm$ 16.3		361.4 $\pm$ 13.3	NS	406.0 $\pm$ 17.1	NS	347.1 $\pm$ 15.5	283.7 $\pm$ 11.1 ^c
(mg/100 g of body wt)	151.5 $\pm$ 6.6		142.4 $\pm$ 5.4	NS	161.1 $\pm$ 6.3	NS	145.9 $\pm$ 6.1	205.3 $\pm$ 10.0 ^c

^c Duration of experiment = 14 weeks.^e Mean $\pm$ SE.^a p values compared to controls: ^a $p < 0.05$; ^b $p < 0.025$; ^c $p < 0.01$; ^d $p < 0.005$; ^e $p < 0.001$; NS = not significant.^d Based on number of nuclei of acinar cells per unit area (approx 0.0025 mm²).

nificant decrease in relative number and diameter of granular tubules but no significant change in the sizes of acinar cells of submandibular glands.

Table III shows that both mean absolute and relative weights of the ovaries as well as the uteri decreased proportionately with increase in daily doses of norethindrone. The absolute and relative thyroid and adrenal wet weights of all norethindrone-treated groups of rats were essentially the same (except the mean relative adrenal weight of rats treated with 60 μ g of norethindrone decreased significantly) as the controls. Estriol treatment resulted in a highly significant decrease in absolute weight of ovaries, uteri, thyroid and adrenal glands, while their relative weights (except that of the ovaries) increased significantly as compared to the controls.

Discussion. Norethindrone (in different dose levels) exerted, in general, a stimulating effect on the growth and development of the submandibular glands with no change in parotid weight. However, it caused an increase in the incidence of carious lesions. The stimulating effect of norethindrone on submandibular glands was possibly due to its gestogenic, androgenic, and anabolic activities. The norethindrone-induced increase in the formation of carious lesions probably was not due to a decrease in the quantity of saliva secretion. However, in these norethindrone-treated rats there might have been changes in quality of the saliva which were favorable to the growth or metabolism of cariogenic microorganisms. Therefore, studies of the chemical composition and physical characteristics of the saliva in norethindrone-treated rats may contribute definitive information on the relationship between salivary glands and carious lesion formation in these treated rats. It also would be of interest to study whether or not thyroxine would synergize the stimulating effect on submandibular gland growth and/or reduce the incidence of carious lesion formation in norethindrone-treated rats.

Since the treatments were begun before the animals reached their puberty, which usually occurs at about 43 days of age for

TABLE III. Effect of Norethindrone on Reproductive Organs, Adrenal and Thyroid Glands in Female Rats.¹

		Vehicle		Norethindrone			Estriol	
	(μ g):			30	60	90		
Wt	No. of rats:	(14)		(16)	(16)	(14)	300	
							(18)	
Ovaries	(mg)	67.8 $\pm$ 2.6		60.3 $\pm$ 2.5 ^a	44.0 $\pm$ 3.4 ^a	26.9 $\pm$ 2.4 ^a	8.9 $\pm$ 0.5 ^a	
	(mg/100 g of body wt)	27.1 $\pm$ 0.8		23.8 $\pm$ 0.9 ^b	16.2 $\pm$ 1.6 ^a	11.3 $\pm$ 0.9 ^a	6.4 $\pm$ 0.4 ^a	
Uterus	(mg)	378.0 $\pm$ 24.1		282.0 $\pm$ 17.6 ^d	188.0 $\pm$ 6.4 ^a	156.7 $\pm$ 5.9 ^a	268.6 $\pm$ 7.5 ^a	
	(mg/100 g of body wt)	151.7 $\pm$ 10.3		111.4 $\pm$ 7.2 ^d	77.4 $\pm$ 3.6 ^a	66.0 $\pm$ 2.5 ^a	188.8 $\pm$ 6.5 ^a	
Thyroid	(mg)	11.3 $\pm$ 0.52		10.8 $\pm$ 0.36 NS	10.6 $\pm$ 0.24 NS	10.2 $\pm$ 0.37 NS	8.5 $\pm$ 0.22 ^a	
	(mg/100 g of body wt)	4.5 $\pm$ 0.20		4.3 $\pm$ 0.14 NS	4.2 $\pm$ 0.10 NS	4.4 $\pm$ 0.14 NS	6.5 $\pm$ 0.39 ^a	
Adrenal	(mg)	60.7 $\pm$ 1.6		60.5 $\pm$ 1.6 NS	55.3 $\pm$ 2.2 NS	60.3 $\pm$ 1.1 NS	39.4 $\pm$ 2.7 ^a	
	(mg/100 g of body wt)	24.3 $\pm$ 0.61		23.9 $\pm$ 0.84 NS	22.0 $\pm$ 0.84 ^a	25.2 $\pm$ 0.44 NS	29.7 $\pm$ 0.64 ^a	

¹ These data are from the same groups of rats as shown in Table II. All footnotes from Table II are applicable.

Sprague-Dawley rats, it was possible that norethindrone might have interfered directly or indirectly, in an unknown manner, with the maturation, chemical composition, or physical properties of the molar teeth rendering them more susceptible to carious attack. Thus, it is desirable to study these parameters of the molar teeth in the treated rats. It also would be of interest to determine whether or not norethindrone would exert a cariogenic effect in adult female rats.

Norethindrone itself does not exert estrogenic or corticoid activities which have been reported as cariogenic in rats (1-4). Furthermore, it caused a decrease in urinary estrogen excretion (9) and a depression in ACTH-induced increase in plasma adrenocortical hormones (10). These were corroborated by the highly significant decrease in ovarian weight and the absence of growing ovarian follicles and no weight change in adrenal glands in norethindrone-treated rats in the present studies. Therefore, norethindrone-induced increase in carious lesion formation were probably not related to an increase in estrogenic or adrenocortical hormones.

Although no observations were made on the effect of norethindrone on plasma protein-bound iodine, norethindrone in combination with mestranol was reported to increase in protein-bound iodine in human beings (11). Still, the thyroid weights, of the treated rats were the same as the controls. Thus, the increased incidence of carious lesion formation in the norethindrone-treated rats was probably not caused by decreased thyroid activity which ordinarily facilitates the formation of carious lesions (6).

A slight to a significant increase in the gain of body weight was observed in rats receiving low dose levels (30 or 60 μ g) of norethindrone. However, at a higher dose level (90 μ g), norethindrone caused a significant decrease in body weight gain without interfering with the growth and development of the submandibular gland. It appeared to be that the submandibular glands were less suscepti-

ble to the toxic dose of norethindrone.

Summary. Weanling female Sprague-Dawley strain rats were divided randomly into groups and were given daily subcutaneous injections of 30, 60, or 90 μ g of norethindrone in 0.1 ml of steroid diluent for 9 or 14 weeks. Norethindrone in different dose levels caused, in general, a slight increase in wet weight and a significant increase in the diameter and relative number of granular tubules and in the sizes of acinar cells of submandibular glands. No significant changes in parotid gland weight were observed. Norethindrone in different dose levels caused an increase in the incidence of carious lesions, but no differences in severity of carious lesions were observed between norethindrone-treated groups of rats and their controls.

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