

Endocrine Factors Affecting Weight and Ascorbic Acid Content of Rat Preputial Glands.* (19355)

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The preputial glands of the rat have generally been considered as accessory sexual organs whose size and function were dependent upon androgenic stimulation(1). Noble and Collip(2), however, suggested a direct effect of pituitary extract on preputial glands in addition to an indirect stimulation by corticotrophic and gonadotrophic principles. Jacot and Selye(3) reported that commercial preparations of adrenocorticotrophic hormone (ACTH) induced enlargement of the preputial glands of adrenalectomized-ovariectomized rats, which represented either an extra-adrenal activity of ACTH or a contaminating pituitary principle present in ACTH. Histochemical studies of preputial glands indicate the presence of ascorbic acid and sudanophilic lipids in the acinar cells, and steroids in the sebaceous secretory product(4). Because of these similarities to the adrenal glands, the present studies were undertaken to determine the normal ascorbic acid content of the preputial glands and to study various factors which affect their weight and vitamin content.

Methods. Exp. I. Thirty-four male rats of the Holtzman strain were adrenalectomized at the age of 33 days, and maintained for 3 weeks on 1% NaCl in the drinking water. Nine of these received .15 mg desoxycorticosterone acetate (DCA) daily, subcutaneously. Ten intact rats served as controls. Exp. II. Eighteen male rats were thyroidectomized at the age of 33 days and 11 intact rats were made hyperthyroid by addition of thyroid powder (0.5% Armour thyroid powder, by weight) to their diet. Twenty untreated rats of the same age served as controls. After 6 weeks, half of each group were stressed by immersing the animal (except the head) in water at 70°C for 5 seconds while under nembutal anesthesia, and autopsied one hour later. The other rats of each group were

sacrificed without stress. The weights of the adrenal and preputial glands were recorded, and ascorbic acid determinations were made on each, using the method of Roe and Kuether (5). Exp. III. Thirteen male rats weighing 120-160 g, 6-7 weeks of age, were hypophysectomized. Twenty-four hours later the left adrenal and left preputial glands were removed, ACTH was injected by tail vein, and one hour later the right adrenal and right preputial glands were removed. The glands were cleaned, weighed on a torsion balance, and ascorbic acid content was determined.

Results. Exp. I. Single preputial glands of adrenalectomized rats, maintained on 1% NaCl in the drinking water, weighed 27.7 mg/100 g body weight, in contrast to a weight of 20.5 mg/100 g body weight for those of intact rats of the same age and sex (Table I). Daily injection of a maintenance dose of DCA to adrenalectomized rats resulted in preputial gland weight of 23.3 mg/100 g body weight.

Exp. II. Hypothyroidism for a period of six weeks following thyroidectomy resulted in a slight increase in preputial gland weight which was not statistically significant. Hyperthyroidism induced a more marked hypertrophy (Table I). Stress produced no statistically significant change in preputial gland weight. Ascorbic acid content of the control preputial glands was approximately 60 mg %, with no significant difference in those rats which were hypothyroid (65 mg %), or hyperthyroid (55 mg %). However, as can be seen in Table I, severe stress decreased the preputial gland ascorbic acid content of the controls by 27%; as compared with 89% in hypothyroid rats and 44% in hyperthyroid rats.

Exp. III. Rats hypophysectomized 24 hours previously showed an ascorbic acid content of the left preputial gland which was 78% that of controls (Table II). One hour after intravenous injection of various doses of ACTH (Armour LA-1A) to such rats, analysis

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TABLE I. Effect of Adrenal and Thyroid Function and Stress on Weight and Ascorbic Acid Content of Preputial Glands.

Treatment	No. of rats	Left preputial wt $\pm$ S.E. (mg/100 g body wt)	Ascorbic acid $\pm$ S.E. (mg/100 g tissue)
Control (8 wk old)	10	20.5 $\pm$ 1.6	—
Adrenalectomized (3 wk)	25	27.7 $\pm$ 1.5*	—
Adrenalectomized (3 wk) + .15 mg DCA/day	9	23.3 $\pm$ 2.2	—
Control (11 wk old)	10	18 $\pm$ 1.7	60 $\pm$ 2.8
Control, stressed†	10	17.1 $\pm$ 2.3	44 $\pm$ 7.7
Thyroidectomized	9	23.9 $\pm$ 2.2	65 $\pm$ 3.9
Thyroidectomized + stress†	9	15.3 $\pm$ 2.2	7 $\pm$ 3.8‡
Thyroid-fed	5	29.8 $\pm$ 3.2*	55 $\pm$ 3
Thyroid-fed + stress†	6	25 $\pm$ 4.1	31 $\pm$ 7 ‡

* Difference from controls statistically significant.

† Immersed in water at 70°C for 5 sec.

‡ Difference from non-stressed groups statistically significant.

TABLE II. Effect of Intravenous ACTH on Weight and Ascorbic Acid Content of Preputial Glands of Hypophysectomized Rats.

Treatment	No. of rats	Preputial wt $\pm$ S.E. (mg/100 g body wt)	Ascorbic acid $\pm$ S.E. (mg/100 g tissue)
Hypophysectomized (24 hr)	13	20.8 $\pm$ 1.3	47 $\pm$ 3
" + .25 μ g ACTH	1	18.9	50
" + 1	3	26.8 $\pm$ 2.9	51 $\pm$ 8.1
" + 2	3	19.7 $\pm$ 1.2	56 $\pm$ 7.7
" + 4	3	21 $\pm$ 3.8	32 $\pm$ 5.8

of ascorbic acid content of the right preputial was made. The only appreciable effect was noted after the highest dose (4 μ g/100 g body weight), which produced a 32% decrease from that of the uninjected hypophysectomized rats.

Discussion. Gemzell *et al.*(6) have shown that adrenalectomy in the rat is followed by more than a 30-fold increase in circulating ACTH. The results in the present adrenalectomy experiment indicate that preputial enlargement is produced by endogenous ACTH hypersecretion comparable to that observed by Jacot and Selye(3) following treatment of rats with commercial ACTH preparations. This makes it unnecessary to assume the presence of a preputial-stimulating contaminant in such preparations, although it does not preclude the possibility. Administration of DCA to adrenalectomized rats impaired the preputial hypertrophy which suggests that this steroid may have partially inhibited the ACTH hypersecretion. Increased weight of the preputial glands in hyperthyroid rats may be explained either on the basis of increased ACTH production(7) or increased androgen

secretion from hyperactive interstitial cells of the testes(8). The statistically insignificant reduction of preputial weight in certain groups one hour after stress may be partly due to extrusion of pre-formed sebaceous secretion in response to sudden sensory stimulation. Actual cellular degeneration seems unlikely.

Ascorbic acid depletion in preputial glands following stress in normal, hypo- and hyperthyroid rats parallels the depletion of adrenal ascorbic acid. Simultaneous determinations made on adrenal glands of the same animals after treatment and reported in another communication(9), show depletions of ascorbic acid in adrenals as follows: normal, 38%; hypothyroid, 75%; and hyperthyroid, 31%. This can be compared to the preputial ascorbic acid depletion of: normal, 27%; hypothyroid, 89%; and hyperthyroid, 44%. It is apparent that in hypothyroidism a much greater depletion of ascorbic acid occurs in response to severe stress(10). This phenomenon of ascorbic acid depletion may be a generalized vitamin depletion, or it may be associated with steroid production. Histochemical studies of Montagna and Noback

(4) locate ascorbic acid in the cytoplasm of acinar cells, along with sudanophilic lipids. Since the aged sebum contains steroids, the preputial glands may be a more favorable site for elucidation of the relationship between ascorbic acid and steroid formation than the more complex adrenal glands.

The preputial content of ascorbic acid is lower in rats hypophysectomized 24 hours previously than in controls. This may be a result of depletion due to trauma associated with the operation without return to the normal level, or may be a result of decreased pituitary hormones.

Summary. 1. The normal level of ascorbic acid in the preputial glands of the young male rat is 60 mg %. 2. Adrenalectomy (3 wks) induces preputial gland hypertrophy, which is partially prevented by DCA. 3. Severe stress produces a depletion of preputial ascorbic acid, which is accentuated in hypo-

thyroid animals. 4. Hypophysectomized rats (24 hr) have a level of preputial ascorbic acid which is 78% that of controls.

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Metabolism of Renal Cortex in Nephrotic Syndrome of Rats.* (19356)

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The chronic renal disease induced in rats by the intravenous injection of nephrotoxic sera obtained from rabbits(1) simulates the nephrotic syndrome as it is observed in infants and children(2). The site of the antibody-antigen reaction is in the renal cortex(3,4), probably in the basement membrane of the glomerulus(5,6). The initial injury elicits a chronic disease that may continue for 12 to 15 months or longer, without further injection of nephrotoxic antibodies.

It is impossible that heteronephrotoxins may have this prolonged effect by virtue of their continued presence. It appears more likely that the initial antibody-antigen reaction produces a cellular injury that may be

irreversible. It thus seemed of interest to investigate whether this lesion is associated with metabolic alterations in the renal cortex of rats in which this disease had been induced.

Methods. Rats of the Long-Evans and Whelan strains were used. They were kept on Friskies and water. Renal disease was induced according to the technic previously described(2) when they were 4 to 5 weeks of age. Animals were chosen that had a well established disease for various lengths of time. Fourteen had had renal disease for less than 8 days, 5 for 1-4 weeks, 14 for 1-6 months, and 9 for 6-14 months. They were sacrificed by decapitation, blood being obtained for chemistry studies. One-fourth to one-half of each kidney was preserved for histological examinations. Immediately thereafter the remaining kidneys were freed of non-cortical tissue and placed in ice-cold Krebs-Ringer

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