livers of the deficient animals resulted in restoration of most of the cysteine desulfhydrase activity. 4. The implications of these findings in terms of the participation of vit. B_6 in the glutamine-α-keto acid reactions are discussed.

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Response of Preputial and Adrenal Glands of the Rat to Sex Hormones.* (20101)

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Paired preputial glands are present in both male and female rats. They are considered accessory reproductive glands, since it is well known that they are stimulated by androgens and to some degree by other steroid hormones (1). Indeed, since they are well developed in both sexes, their weight has often been used as a measure of the endogenous androgen production of gonads or adrenal glands(2). Only recently has it become apparent that in certain aspects the response of the preputial glands to ACTH parallels that of the adrenal glands. Thus, highly purified preparations of ACTH are reported to exert a direct, growth-promoting action on the preputial glands of adrenalectomized-ovariectomized rats(3,4). Furthermore, such a growth response need not be attributed to a contaminating principle present in the ACTH preparation, since it has also been shown that preputial gland hypertrophy follows adrenalectomy, an operation known to evoke a hypersecretion of ACTH(5). It appears, therefore, that the direct action of ACTH is not limited to the cells of the adrenal cortex, but extends also to those of the preputial glands. Such a concept is in accord

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with the recent demonstration that either severe stress or intravenously administered ACTH produces a rapid fall in the level of preputial ascorbic acid(5), and with the fact that the preputial and adrenal glands show a similar response as regards weight and ascorbic acid level after hypophysectomy and following either acute or chronic administration of ACTH to hypophysectomized rats(6).

Much is already known about the effects of sex hormones on the weight of both of these glands but there is little information as to what effect these hormones have on the ascorbic acid content of either adrenal or preputial glands. It is currently thought that androgens cause a diminution in adrenal weight when given to rats of either sex, whether intact or gonadectomized(7). In contrast, estrogens induce adrenal hypertrophy in the rat, this effect being dependent upon the presence of the pituitary gland. Since both androgens and estrogens are believed to influence the weight of the preputial glands, it seemed of interest to make a comparative study of the effects of these hormones on the weight and ascorbic acid content of the preputial and adrenal glands. In addition, a study was made of the direct action of androgen treatment on the preputial and adrenal glands of the hypophysectomized rat as well as the effect of such a treatment on the responsiveness of these glands to the ascorbic acid-depleting action of ACTH.

Methods. Forty-five male rats of the Holtzman strain were divided into 3 equal groups when 30 days of age. All animals were kept in air conditioned quarters and given laboratory chow (Purina) and tap water. The animals in one group received daily, subcutaneous injections of 2.5 mg testosterone propionate, while those in the second experimental group were given subcutaneous injections of 50 μg estradiol benzoate daily. The third group served as untreated controls and all animals were killed 40 days after the injections were begun. In order to obviate any effects of pituitary stimulation the animals were quickly decapitated. The left adrenal and preputial glands were removed and after freeing them of fat and connective tissue, they were weighed on a torsion balance and their ascorbic acid

TABLE I. Effect of Estrogen and Androgen on Adrenal and Preputial Glands of Intact and Hypophysectomized Male Rats.

	No. rats	Adrenal glands				Preputial glands			
		Wt, mg		Ascorbic acid content, μg		Wt, mg		Ascorbic acid content, μg	
		Abs	Rel†	Abs‡	Rel§	Abs	Rel†	Abs‡	Rel§
Controls	13	18.2 ± .4¶	7.0 ± .2	82 ± 5.6	438 ± 21	54.4 ± 5.4	21.0 ± 2.2	29 ± 2.4	57 ± 3.4
Estrogen-treated	13	22.3 ± 1.0	14.0 ± .6	96 ± 6.1	388 ± 5.2	26.2 ± 1.9	16.5 ± 1.0	19 ± 2.2	81 ± 7.8
Androgen-treated	11	19.9 ± .6	8.8 ± .3	68 ± 6.0	342 ± 29	93.2 ± 13.0	42.0 ± 5.9	28 ± 3.4	33 ± 2.5
Hypophysect. (3 wk)	14	5.4 ± .2	5.1 ± .2	13 ± .6	247 ± 11	9.7 ± .5	9.1 ± .5	3 ± .9	24 ± 7.2
Hypophysect. (3 wk) + androgen*	17 L	6.1 ± .3	5.4 ± .2	11 ± .7	187 ± 14	42.4 ± 2.7	37.1 ± 2.2	6 ± .7	13 ± 1.5
	R‖	5.6 ± .3	5.0 ± .2	6 ± .8	106 ± 9.7	37.8 ± 3.4	33.1 ± 2.8	6 ± 1.0	16 ± 3.3

* 1.25 mg testosterone propionate inj. subcut. daily for 3 wk.
† mg/100 g body wt.
‡ μg ascorbic acid in entire gland.
§ μg ascorbic acid/100 mg tissue.
‖ 10 μg ACTH inj. intrav. 1 hr prior to autopsy.
¶ Standard error.

TABLE II. Effect of Estrogen and Androgen Treatment, Hypophysectomy and Hypophysectomy plus Androgen Treatment on Body, Testis and Prostate Weight of Rats.

	No. rats	Body wt, g		Left testis wt, mg		Ventral prostate wt, mg	
		Initial	Final	Abs	Rel†	Abs.	Rel†
Controls	13		260±6.6‡	1519±42	584±10.1	301 ±26	114 ± 1.0
Estrogen-treated	13		159±3.5	161± 7.8	103± 7.7	15.7± 1.0	9.2± .6
Androgen-treated	11		226±5.5	1237±25	549±15.4	856 ±43	378 ±16
Hypophysect. (3 wk)	14	122±1.7	109±1.8	206±12	191±11	9.5± .6	8.8± .5
Hypophysect. (3 wk) + androgen*	17	115±1.1	114±2.8	912±42	801±34	472 ±18	416 ±14

* 1.25 mg testosterone propionate inj. subcut. daily for 3 wk.
† mg/100 g body wt.
‡ Standard error.

content determined by the method of Roe and Kuether(8). Weights were also recorded for the left testis and ventral prostate of all animals. Thirty-one male rats of the Holtzman strain were hypophysectomized at the age of 33 days. These animals were maintained on laboratory chow and 5% sucrose solution during the post-operative period. Following hypophysectomy 17 of the animals were given daily. subcutaneous injections of 1.25 mg testosterone propionate. At the end of 3 weeks the left adrenal and preputial glands were surgically removed, using nembutal anesthesia. These glands were weighed and analyzed for ascorbic acid. Fourteen of these animals were then given an intravenous injection of 10 μg ACTH.‡ One hour following the ACTH injection, the animals were autopsied and the right adrenal and the right preputial glands removed, weighed and analyzed for ascorbic acid. As in the previous experiment the weight of the left testis and ventral prostate was recorded for each animal. The left adrenal and preputial glands were surgically removed from the remaining 14 animals at 3 weeks after hypophysectomy. The effect of an intravenous injection of ACTH on the ascorbic acid of the right glands was then determined. The results of this experiment have been previously reported(6) and only the values for the left glands are repeated here for the purpose of comparison. Testis and ventral prostate weights are likewise repeated for the animals in this group.

Results and discussion. The results of these experiments are summarized in Tables I and II. Due to the pronounced effect of the hormones on body weight, the weights of adrenal and preputial glands are recorded on a relative as well as on an absolute basis. For similar reasons, since gland weights were highly modified in certain groups, the ascorbic acid content has been calculated on both an absolute (μg in entire gland) and relative basis. This enables one to determine if the observed changes in the concentration of ascorbic acid are simply a reflection of the dilution or concentration effect imposed by hypertrophy or atrophy of the gland.

It is apparent that estrogen treatment causes a profound hypertrophy of the adrenal gland, the mean for this group being 100% more than that of the untreated, control animals. This is in agreement with previous reports in the literature(9-11) and according to present concepts is due to an increased secretion of ACTH. In direct contrast to this, we find that the preputial glands of the estrogen-treated group weigh much less than those of the control animals. Even on a relative basis, they are somewhat lighter, although when calculated in this manner the difference is not significant. In making these comparisons, it is well to bear in mind that the preputial glands are directly stimulated, not only by ACTH, but presumably also by androgens. In this regard, it is evident from the testis and ventral prostate weights of the estrogen-treated group that these animals are virtually hypophysectomized, insofar as gonadotropic hormone production and in turn androgen production is concerned. It is perhaps important, then, to notice that whereas the preputial glands are much reduced in weight in

‡ Armour ACTHAR (dosage expressed as LA-1A equivalent).

the estrogen-treated animals they are yet significantly heavier than those of animals which have been hypophysectomized for 3 weeks. It is conceivable that their weight represents the maximum that can be maintained over a long period of time by ACTH in the absence of androgen. This is in accord with the results obtained after treating hypophysectomized rats with ACTH(6).

The changes in the ascorbic acid content of the preputial and adrenal glands observed in the estrogen-treated animals can be largely accounted for in terms of the pronounced weight changes of these organs. Thus, the enlargement of the adrenal glands is accompanied by an increase in the total amount of ascorbic acid per gland. The latter is proportionately somewhat smaller than the increase in weight so that the concentration of ascorbic acid is less in the estrogen-treated than in the control adrenals. In contrast, there is a fall in the total amount of ascorbic acid in the preputial glands, but since this decline does not keep pace with the loss in weight, the concentration remains somewhat higher than in control glands. If one considers only the values for the concentration of ascorbic acid in these glands, it appears that estrogen treatment produces a reduction in the level of adrenal ascorbic acid but an increase in the level of preputial ascorbic acid. In such long-term experiments, however, such over-simplified interpretations seem unwarranted. Assuming that we are dealing with an estrogen-induced hypersecretion of ACTH, our results are compatible with those of Gordon(12) who found that continued daily injections of ACTH to rats for periods of several weeks was without effect on the level of their adrenal ascorbic acid.

It will be noted that the adrenal glands of the testosterone propionate-treated rats are heavier than those of the controls, either in terms of absolute or relative weight. This increase in adrenal weight is significant ($P < 0.02$). Although it is generally agreed that androgens inhibit the adrenal hypertrophy which follows castration of the male rat(13) data relating to the effect of androgen treatment on the adrenal weight of the intact male rat are fragmentary and completely unsatisfactory. The few papers which deal directly with this question indicate that androgen treatment is without any very striking effect on the adrenal weight of intact male rats (14-17). In some cases (as in the data of Korenchevsky and Hall, 1939) where it is possible to calculate relative adrenal weights a tendency for androgen treatment to produce adrenal hypertrophy is apparent. Our results leave no doubt that adrenal hypertrophy may result from androgen treatment although they do not preclude the possibility that under different experimental conditions (*i.e.*, factors such as dosage, length of treatment, age of animals, etc.) a qualitatively different outcome might result.

The literature is equally confusing in regard to the direct action of androgens on the adrenal glands of the hypophysectomized rat. Several workers have made the claim that when injections are begun at the time of hypophysectomy a partial maintenance of adrenal weight can be realized(18-21). Of the more recent papers on this subject only one by Lewis, DeMajo, and Rosemberg(22) reports a further diminution in adrenal weight as a result of androgen treatment. This effect was obtained in hypophysectomized female rats following the daily injection of either testosterone propionate (3 rats) or 17-vinyl testosterone (3 rats) for a 20-day period. The results obtained in the present study show that 1.25 mg testosterone propionate daily has no significant effect on the relative adrenal weight of the hypophysectomized male rat. It can be seen, however, that there appears to be a slight maintenance effect if only the absolute adrenal weight is considered, due to the tendency for the androgen treatment to prevent the loss in body weight otherwise seen after hypophysectomy. No doubt this general maintenance effect accounts, at least in part, for what has been regarded as a direct action of androgen on the adrenal. Thus, the data of Zizine, Simpson, and Evans(21) are much less striking if the adrenal weights are calculated on a relative basis. Even so, it is difficult to reconcile their results with those obtained in the present study. Analysis of their data indicates not only that androgen has a specific adrenal weight-maintaining

effect, but (contrary to their own conclusions) that this effect is at least in part dependent on the presence of the gonads. Since the dosage they employed was double that used in the present study, this may prove to be a valuable lead in the solution of this important problem. Turning now to the effect of androgens on the weight of the preputial glands, it is apparent that testosterone propionate is capable of producing marked hypertrophy of these glands in either the intact or hypophysectomized rat. This proves that androgen exerts a direct, growth-promoting action on the preputial glands although it is reasonable to suppose that in the intact animal androgens may also modify the size of these glands by virtue of their capacity to alter the rate of ACTH secretion. As a matter of fact, it seems likely that the estrogens as well as progesterone may affect preputial size chiefly in this manner. It is interesting to note that the relative size of the preputial glands as well as the ventral prostate is approximately the same in the intact and hypophysectomized androgen-treated groups, which further emphasizes the fact that the action of androgen on the preputial glands is predominantly a direct one.

It should also be pointed out that our results confirm previous reports(7) that androgen treatment will effectively maintain the weight of the testes of the hypophysectomized rat. Examination of histological sections of such testes shows the tubular epithelium to be essentially normal.

The situation with regard to the ascorbic acid in the adrenal and preputial glands is quite a different one than was seen after estrogen treatment. Thus, in considering the preputial gland, it is clear that androgens serve to enlarge the gland but do not produce a corresponding increase in ascorbic acid. This is seen in both the intact and hypophysectomized groups. By comparison, androgen treatment can apparently produce a diminution of adrenal ascorbic acid in the absence of any appreciable change in adrenal weight. This is seen in the intact groups where there is a significant difference in the concentration of ascorbic acid in the adrenals of the androgen-treated rats as compared with those of the controls. The same is true in the hypophysectomized groups where androgen treatment further reduces the already low level of adrenal ascorbic acid.

Finally, the effect of androgen treatment on the responsiveness of the adrenal and preputial glands to the ascorbic acid-depleting action of a single, intravenous injection of ACTH was determined. In a previous publication(6) it was pointed out that such an intravenous injection of ACTH would cause a precipitous fall in the level of ascorbic acid in the preputial and adrenal glands of the hypophysectomized rat. A comparable drop in the level of adrenal ascorbic acid can be observed in the present study at one hour after the intravenous injection of 10 μg ACTH into androgen-treated, hypophysectomized rats. In contrast, no such reduction in the level of the preputial ascorbic acid of these animals was observed. This indicates that testosterone propionate, in some manner, destroys the responsiveness of the preputial gland to the ascorbic acid-depleting action of ACTH.

Summary and conclusions. The administration of estrogen to intact male rats for 40 days induces hypertrophy of their adrenal glands but has the reverse effect on their preputial glands. The latter effect is undoubtedly a result of the virtual cessation of endogenous androgen production. Alterations in the ascorbic acid content of these glands appear to be a reflection of the marked changes in their weight. Androgen treatment for the same period produces a slight but significant increase in the weight of the adrenal glands and a 2-fold increase in preputial gland weight. This effect on the preputial glands is predominantly a direct one, as it is seen also in hypophysectomized animals. In contrast, no direct action of androgen on the weight of the adrenal glands of hypophysectomized rats was observed. It was found that androgen administration reduces the resting level of ascorbic acid in the adrenal and preputial glands of both intact and hypophysectomized rats. Furthermore, this hormone somehow destroys the responsiveness of the preputial glands to the ascorbic acid-depleting action of a single injection of ACTH.

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Angiostomy Cannulae for the Study of Pulmonary Circulation.*† (20102)

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London(1) used angiostomy cannulae for the purpose of directing hypodermic needles into the larger thoracic vessels. Daly(2) reported the measurement of pulmonary arterial pressure in the unanesthetized dog by means of the London Type cannulae. Johnson, Hamilton, Katz and Weinstein(3) in the same year studied the dynamics of pulmonary circulation by means of hypodermic manometers, the needles being placed in the aorta, pulmonary artery and pulmonary vein. Hamilton, Woodbury and Vogt(4) studied differential pressures in the lesser circulation of the unanesthetized dogs using angiostomy cannulae and hypodermic manometers. Katz and Steinitz(5) developed a modification of the angiostomy cannulae used by Hamilton, and used their cannulae in measuring pulmonary arterial pressures.

The purpose of this paper is to report the development of a technic permitting direct access to the atria and large thoracic vessels of unanesthetized dogs under conditions which may be compared to normal.

Methods. Two varieties of cannulae were developed and used. The first variety, shown in Fig. 1, a., consists of silver tubes (3 mm outside diameter) fitted with perforated silver plates, one type of which is trough-shaped, to fit over the left pulmonary artery. A second type is flat and oval in shape, suitable for attachment to the left atrium. The length of each silver tube is such that its distal end, the heart serving as reference point, will lie immediately under the skin of the chest wall. The perforated silver plates are made from 2 mm silver stock. The plate attachment that is fitted over the left pulmonary artery is 8 mm long and of sufficient width so that when bent in a semicircle of the correct radius it extends over half the circumference of the

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