Influence of Sex on Adrenal Regeneration and Accompanying Hypertension. (23174)

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Skelton and Guillebeau(1-3) have reported that rats subjected to right nephrectomy, right adrenalectomy and left adrenal enucleation, demonstrated regeneration of adrenal tissue accompanied by hypertension and characteristic lesions. In this laboratory female rats so prepared (henceforth referred to as NAE-rats) yielded results similar to those reported. However, when a group of male rats was prepared in the same manner only 3 of 31 became hypertensive after 9 weeks of observation. The average systolic blood pressure was 125 mm Hg, ranging from 102 to 164 mm Hg, whereas, females averaged 148 mm Hg, ranging from 140 to 160 mm Hg. Control values averaged around 110 mm Hg. Correspondingly heart weights of the hypertensive females were significantly greater than those of the males. Even more significant was the observation that the hypertensive females had regenerated nearly 3 times more adrenal tissue than the normotensive males.

This suggested that sex hormones might be involved, either directly or indirectly, in adrenal regeneration accompanied by hypertension. Failure to elicit a blood pressure rise in male rats was possibly due to an inhibiting effect of androgens. To explore this possibility, 3 approaches were made. First, NAE-female rats were treated with testosterone to determine whether the male hormone might suppress regeneration of the adrenal and hence the subsequent development of hypertension. Second, NAE-hypertensive female rats were ovariectomized and then given daily injections of testosterone propionate. This study was designed to ascertain if an established hypertension could be reversed by radical treatment, *i.e.*, a minimum of estrogen plus an excess of androgen. Third, NAE-males were castrated to reduce endogenous testosterone.

Materials and methods. Rats from the Upjohn colony (Wistar strain), 4 weeks old, weighing 60-65 g were caged individually and maintained on an adequate stock diet and 1% sodium chloride solution *ad libitum*. Beginning at the ninth week and at weekly intervals thereafter, systolic blood pressures were determined using a photoelectric tensometer (4). Following extensive observations among normal and DCA-hypertensive rats it has been the practice in this laboratory to consider pressures above 140 mm Hg as hypertensive. All the animals were sacrificed at 17 weeks of age, necropsied, various organs weighed, and the adrenal glands saved for histologic study. The experimental groups were as follows: *Group I.* 10 female rats as untreated unoperated controls. *Group II.* 10 NAE-female rats were prepared. Starting immediately post-operatively, testosterone propionate (TP) at a dose of 100 μg/rat/day was administered subcutaneously. Six weeks post-operatively the dose was increased to 200 μg. *Group III.* Eighteen NAE-female rats were prepared. Eight weeks post-operatively, when hypertension had developed, this group was divided so that the 2 subgroups of 9 rats each had the same average blood pressure. One sub-group served as hypertensive controls, the other constituted Group IV. *Group IV.* Nine hypertensive rats from Group III were ovariectomized and injected subcutaneously with 200 μg of TP/rat/day. *Group V.* Fifteen males were castrated 3 days before NAE treatment. *Group VI.* Five males castrated only and carried as controls. *Group VII.* Fifteen NAE-male rats were prepared. *Group VIII.* Five male rats were maintained as unoperated controls.

Results. Average systolic blood pressure values over the 13 week experimental period for each group are presented graphically in Fig. 1. In agreement with previous experiments and published reports(1,2), it was found that NAE-female rats (Group III) developed high blood pressure as compared to

TABLE I. Terminal Data on NAE-Prepared Rats Subjected to Various Treatments.

Group No.	No. of rats	Description of treatment	Final blood pressure (mm Hg)	Body wt (g)	Heart (mg/100 g body wt)	Adrenal (mg/100 g body wt)
I	10	Unoperated ♀ controls	106 ± 2	239 ± 6*	315 ± 4	20.6 ± .8†
II	10	NAE-♀ + TP	123 ± 3	281 ± 4	329 ±10	7.8 ± .5
III	9	NAE-♀	150 ± 3	246 ± 8	370 ± 7	16.7 ±1.0
IV	8	NAE-♀ + ovariectomy + TP	129 ± 2	260 ± 5	350 ± 7	12.6 ±1.0
V	15	Castrated + NAE-♂	114 ± 3	301 ± 6	281 ± 6	6.5 ± .3
VI	5	Castrated ♂ controls	109 ± 2	332 ±13	263 ± 8	10.6 ± .7†
VII	15	NAE-♂	124 ± 3	385 ± 6	270 ± 9	5.7 ± .5
VIII	5	Unoperated ♂ controls	107 ± 2	391 ±14	258 ± 2	10.4 ± .3†

* Stand. error of the mean. † Both adrenals.

TP = Testosterone propionate. NAE = Right nephrectomy, right adrenalectomy and left adrenal enucleation.

intact female controls (Group I). In contrast, NAE-male rats (Group VII) had significantly ($P<0.01$) lower blood pressures throughout the experimental period than those of the NAE-females (Group III) and only slightly greater than those of the controls (Groups I and VIII).

From the data presented in Table I, it was seen that heart weights generally correlated with terminal blood pressure values. Terminal adrenal weights confirmed the previous observation that those NAE-rats which developed higher blood pressures also demonstrated the most regeneration of adrenal tissue. Thus, the NAE-female rats (Group III) had single adrenals which weighed nearly as much as both adrenals from the intact females (Group I). With either the NAE-males (Group VII) or the castrated NAE-males (Group V) there appeared to be some adrenal regeneration as the single adrenals had terminal weights equal to about half of the weights of 2 adrenals from the controls (Groups VIII and VI). Histologic examination indicated that limited regeneration had taken place, largely, in the *zona fasciculata*. Blood pressure increases in these groups (Groups V and VII) were correspondingly slight.

Daily administration of testosterone propionate to NAE-female rats (Fig. 1, Group II) had a moderating effect on development of higher blood pressures for 5-6 weeks postoperatively at which time the pressure differences of treated and untreated rats became insignificant. Upon increasing the dose of TP 2-fold the systolic blood pressure of the treated group was again depressed significantly ($P<0.01$) below the untreated group (Group III) for the duration of the experiment. The smaller average heart weight (Table I) of the TP-treated rats (Group II) confirmed the blood pressure observations; regeneration of adrenal tissue was found to be less than half that of the untreated NAE-females (Group III).

NAE-females which had become hypertensive and then subjected to ovariectomy plus daily TP administration for 30 days (Group IV), demonstrated a significant fall in blood pressure (Fig. 1). Other data for this group (Table I) revealed that terminal adrenal weight was statistically smaller than that of

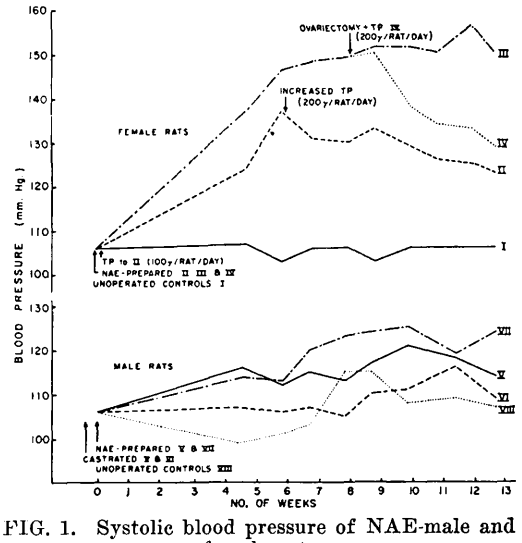

FIG. 1. Systolic blood pressure of NAE-male and female rats.

the untreated controls (Group III) ($P < 0.01$); the enlarged, hypertensive heart had not regressed significantly.

Discussion. Testosterone treatment accompanied with ovariectomy not only reversed an established hypertension of NAE-female rats but also appeared to cause a regression of adrenal mass (Group IV), since, at the time treatment was initiated, the adrenals of Groups III and IV were presumably equal and close to maximal size. It has been reported(5) that testosterone provoked adrenal atrophy when administered to female rats under other experimental conditions. However, reduction of endogenous testosterone by castration of NAE-male rats did not produce high blood pressure even though all NAE-males showed some adrenal regeneration. It is possible this phenomenon is dependent upon the presence of an adequate concentration of estrogens. Estrogens have been reported to produce adrenal hypertrophy (6) and hypertension in rats(7,8). The response of the castrated NAE-male rat to exogenous estrogen is being investigated.

Summary. Evidence has been presented that NAE-female rats but not NAE-males demonstrated adrenal regeneration accompanied with hypertension. Testosterone administered to NAE-female rats inhibited regeneration of the enucleated adrenal and development of hypertension. Testosterone caused a reduction in the blood pressure of ovariectomized, hypertensive NAE-female rats. Castration was not effective in promoting adrenal regeneration or hypertension in the male rat.

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A Method of Isolating Normal and Fowlpox Infected Chorioallantoic Epithelium.* (23175)

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In this laboratory we are concerned with an investigation of certain chemical changes which appear to be induced in chorioallantoic epithelium infected with fowlpox virus. Goodpasture(1) has shown that in fowlpox infection the hyperplastic ectoderm contains numerous large inclusions, that to a lesser degree the entoderm is involved as well, and that there is accompanying thickening of the mesoderm. It would be very desirable to obtain infected epithelium in quantity without inclusion of mesodermal elements which may tend to obscure the results.

Procedure. Three different batches each consisting of 3-6 dozen embryonated hen eggs were inoculated with fowlpox on the chorioallantoic membranes by the Goodpasture method(2) and incubated for 5 days at 37°C. A comparable number of uninoculated control eggs were incubated under the same conditions. Infected and control membranes were collected aseptically in cold physiological saline and refrigerated for several hours until

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