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Effect of Time of Reduction of Renal Mass on Development of Adrenal-Regeneration Hypertension.* (29899)

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Reduced renal mass often is associated with clinical hypertensive disease, and it has been demonstrated by Selye(1) that unilateral nephrectomy enhances the hypertensive potential of steroids in the rat. Skelton(2,3) has shown that uninephrectomy enhances the hypertension which develops during adrenal regeneration in the rat. Although the exact role which the reduction of renal mass plays in production of adrenal-regeneration and steroid hypertension is not understood, one hypothesis holds that it may be through reduced renal capacity to handle sodium.

The present paper deals with the investigation of the effect which time of nephrectomy might have on the development of the experimental syndrome of adrenal-regeneration hypertension.

Materials and methods. Weanling female Sprague-Dawley rats were used in these experiments. All rats were individually caged and fed Purina Lab Chow and given 1% sodium chloride solution to drink *ad libitum*.

Groups of rats were set up as shown in Table I. Saline intake was measured throughout the experiment and blood pressures of the rats were taken at weekly intervals using the microphonic method of Friedman and Freed (4). After 7 weeks the rats were sacrificed by decapitation and organs were dissected, examined for gross lesions, weighed and fixed in formalin. Sections were prepared and stained with hematoxylin and eosin for microscopic examination of the lesions. Severity of the gross and microscopic changes in the kidney, heart, brain and mesenteric vascular bed was graded on a zero to 3+ scale. Values so derived were summed for each rat and overall gross and microscopic lesion severity indices obtained.

Results. The blood pressure measurements from the various groups are depicted in Fig. 1 and 1a. Simultaneous unilateral nephrectomy, unilateral adrenalectomy and contralateral adrenal enucleation as performed in Group 2 resulted in the well-known adrenal-regeneration hypertension described by Skelton(2). The combination of operative steps used with Groups 3 and 6 also resulted in hypertension, kidney and heart enlargement, and high lesion severity indices. When the same surgical procedure was used as for Group 2 but in animals 2 weeks older (Group 5), there was some reduction in the hypertension. In Group 4, where the renal mass was reduced 2 weeks after the unilateral ad-

TABLE I. Arrangement of Experimental Groups.

Group No.	Operations at 'zero time'*	Operations at 2 wk
1	R. nephrectomy	—
2	R. " R. adrenalectomy L. adrenal enucleation	—
3	R. nephrectomy	R. adrenalectomy L. adrenal enucleation
4	R. adrenalectomy L. adrenal enucleation	R. nephrectomy
5	—	R. nephrectomy R. adrenalectomy L. adrenal enucleation
6	R. nephrectomy R. adrenalectomy	L. adrenal enucleation

* At "zero time" the rats were 24 days old.

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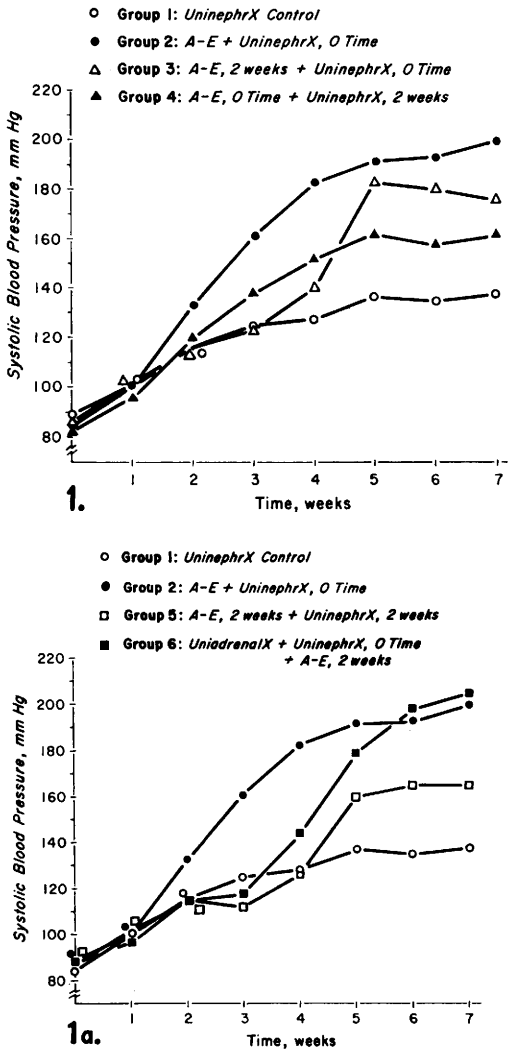

FIG. 1 and 1a. Change of systolic blood pressure with time.

renalectomy and contralateral adrenal enucleation, significantly lower blood pressures were found as compared to Groups 2, 3 and 6. It should be noted that the sequence of operative procedures carried out on Group 4 was the reverse of that done on Group 3 and the mean terminal blood pressure of Group 3 was significantly above that of Group 4 ($p < 0.02$).

Lesion severity indices and organ weights are shown in Table II. The rats in Groups 2, 3 and 6 developed significant cardiac and renal enlargement and correspondingly high gross and microscopic lesion severity indices when compared with uninephrectomized controls (Group 1). In those rats whose blood pressure rose less markedly (Groups 4 and 5), these parameters showed only slight change from control values.

Saline consumption in the various groups is shown in Fig. 2. Despite the similarity of terminal blood pressure levels, the saline consumption pattern of Group 6 is in sharp contrast to that of Group 2 which had an elevated intake throughout the experiment. In Group 6 saline intake increased steeply only after enucleation and surpassed the intake of Group 2 after the fourth week (fifth week, Group 2 *vs* Group 6: $p < 0.05$). The increase in saline intake coincided with the observed increase in blood pressure. The effect of adrenal enucleation is again evident in the comparison of saline intake of Groups 2 and 3. In Group 3 there was little change in saline intake following uninephrectomy but following uniadrenalectomy and adrenal enucleation

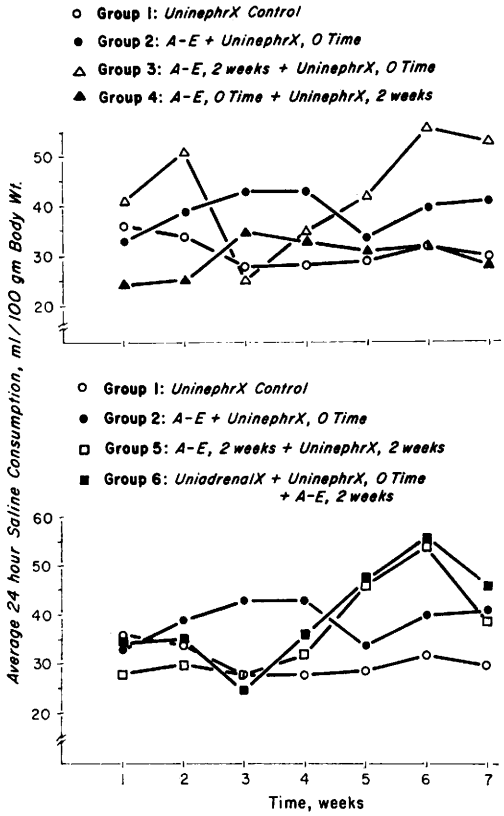

FIG. 2. Saline intake.

TABLE II. Mean Organ Weights, Lesion Severity Indices and Terminal Blood Pressures.

Group	n	Organ wt (mg % body wt)*				Severity index		Terminal B.P. (mm Hg)
		Heart	Kidney	Brain	Spleen	Micro	Macro	
1	9	393 ± 8	814 ± 34	788 ± 16	313 ± 15	.20 ± .20	0	141 ± 4
2	8	597 ± 73	1101 ± 62	1007 ± 81	441 ± 82	1.23 ± .53	1.08 ± .37	204 ± 6
3	10	542 ± 33	1028 ± 59	936 ± 51	394 ± 30	1.37 ± .27	1.17 ± .18	193 ± 7
4	8	430 ± 35	831 ± 89	925 ± 75	322 ± 15	.52 ± .10	.31 ± .11	166 ± 8
5	10	433 ± 10	797 ± 31	798 ± 10	319 ± 14	.65 ± .03	.14 ± .08	173 ± 3
6	10	521 ± 24	1013 ± 60	833 ± 26	361 ± 28	1.65 ± .53	1.30 ± .60	211 ± 5

* Mean ± S.E.M.

at 2 weeks a significant drop in saline intake occurred followed by a sharp increase which lasted throughout the remainder of the investigation.

Saline consumption in Group 4 which remained unchanged during the 2 weeks following adrenal enucleation and uniadrenalectomy, rose slightly yet significantly ($p < 0.05$) after right nephrectomy only to return to the same range as the uninephrectomized controls towards the end of experiment. In Group 5, saline consumption showed little change until the third week, after which it rose in similar fashion to Groups 2 and 6.

Discussion. While the importance of the reduction of renal mass had been recognized for the development of adrenal-regeneration hypertension(2,3) it was not known at what point during the regenerative process this reduction played a critical role and also to what degree the hypertensive-cardiovascular disease could be modified.

The present study indicates that of the various combinations of operative steps originally described by Skelton(2) the one that combines nephrectomy-adrenalectomy with adrenal enucleation (Group 2) is the most effective for production of hypertension and cardiovascular lesions. When the identical operation is performed 2 weeks after zero time (Group 5), blood pressure levels as well as degree of gross and microscopic lesions are significantly less.

That the reduction of renal mass is an essential step in the combination of operative procedures is suggested by the observation that those groups in which nephrectomy was performed 2 weeks after zero time (Groups 4 and 5) showed lower blood pressure rises and lesser lesion severity indices than the groups where uninephrectomy was performed at zero time (Groups 2, 3 and 6).

It is particularly noteworthy that, although in Group 3 uninephrectomy and uniadrenalectomy-adrenal enucleation are separated in time by 2 weeks, blood pressure, organ weights and lesion severity indices are greater than those of Groups 4 and 5 and almost identical with Groups 2 and 6. This suggests that, although reduction of renal mass contributes to the production of adrenal-regeneration hypertension, the adrenal enucleation procedure is the essential step in precipitating the rise of blood pressure.

The remarkable rise of blood pressure and the high cardiovascular lesion severity index in Group 6 show that the presence of only one adrenal, which undergoes hypertrophy between zero time and 2 weeks later, does not materially affect the development of the hypertensive syndrome which follows enucleation of this hypertrophied adrenal.

This result contrasts with the finding of Takeda *et al*(5). These workers did not find an increased saline intake or blood pressure in animals adrenal enucleated 2 weeks after uninephroadrenalectomy. This difference is difficult to explain although the small number of animals used by these authors, the use of different animals to obtain blood pressure and saline intake values at different times post-enucleation and the difference in strain of rat may be contributory.

Summary. 1. Salt-treated, uninephrectomized-adrenalectomized and contralaterally adrenal enucleated female rats in which those operative steps are performed shortly after weaning (zero time) show a higher mean terminal blood pressure and greater enlargement of heart, kidney, brain and spleen than

do similar rats that are operated on 2 weeks after zero time. 2. Performance of the uninephrectomy 2 weeks after uniadrenalectomy and adrenal enucleation results in significantly lower terminal mean blood pressure and correspondingly lower lesion severity indices. This suggests that the enucleation step proper is the crucial operation in bringing about the full severity of the hypertensive syndrome. 3. Uninephrectomy and uniadrenalectomy on day zero and contralateral enucleation of the compensatorily-hypertrophied adrenal 2 weeks later results in mean terminal blood pressure and lesion severity indices comparable to those rats in which these operations are carried out at zero time. Blood pressure only rises steeply after the hypertrophied adrenal gland is enucleated. 4. It is concluded that, while reduction of renal mass contributes greatly to the development of this form of experimental hypertension, enucleation of the adrenal is the decisive step. It is further concluded that this combination of operations early in infancy finds a more favorable internal environment for the development of severe hypertensive cardiovascular disease.

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Use of Tissue Culture Cultivated Toxoplasma in the Dye Test and for Storage.* (29900)

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The successful cultivation of *Toxoplasma gondii,* an obligate intracellular parasite, in tissue culture, has been described in several reports(1-6). Chernin and Weller(1,2) demonstrated that free organisms could be obtained in roller-tube cultures. This communication describes the use of roller-tube cultures of toxoplasma as a source of parasites for performance of the dye test(7) and for long-term storage of viable organisms.

Material and methods. Hep-2 cells were used throughout. The cells were maintained in 2.5% inactivated (30 minutes at 56°C) calf serum in medium 199 to which had been added 10% lactalbumin hydrolysate (LH) and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml, nystatin 50 U/ml).

The tissue cultures were inoculated initially with mouse peritoneal fluid infected with the RH strain of toxoplasma. The first 3 passages were maintained as stationary cultures at 35°C. They were transferred when the cytopathic effect (CPE) was marked and many toxoplasmas were present in wet mounts stained with methylene blue buffered at pH 11(7). Passage was accomplished by pooling several such fluids and transferring 0.1-0.2 ml aliquots to fresh culture tubes. Comparable uninoculated control tubes were maintained and passed in a similar manner. In these stationary cultures 13-17 days were required for the CPE to appear and more than 21 days for a significant number of free parasites to become recognizable.

One-half of the fourth passage tubes were placed in a roller drum revolving at 1/5 rpm. Since large numbers of organisms were observed to be floating free in the medium by the 7th or 8th days (with experience free toxoplasmas could be detected easily at

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