Renal Hypertrophy Factor in Serum of Nephrectomized Rats, with Observations on Species Specificity[1] (34982)

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Compensatory renal hypertrophy after unilateral nephrectomy is well known. Among several explanations for such an occurrence, a factor in the serum of unilaterally nephrectomized rats that causes renal hypertrophy has been described (1–4). However, this hypothesis is not completely accepted, and one report denied the existence of such a factor (5). However, a renal hypertrophy effect has been noted after injection into normal rats of serum from bilaterally nephrectomized animals (6, 7).

The present study was undertaken to add evidence for or against the presence of a renal hypertrophy factor in the serum of uni- and bilaterally nephrectomized rats.

Weight and the uptake of tritiated thymidine of the kidneys of assay (or recipient) rats were utilized in this study (8). Thymidine uptake proved to be the most sensitive and useful indicator. Our data confirmed the presence of such a factor in nephrectomized rats. We were unable to observe the effect in the rat assay of sera obtained from uni- and bilaterally nephrectomized dogs and man, and from a patient with severe renal insufficiency. The renal hypertrophy factor appears to be species specific, although an alternative explanation for our observations is possible.

Materials and Methods. Male albino rats of a Sprague–Dawley strain, ranging in weight from 140–150 g, were divided into three groups:

Group I (rat donors). Eighty recipient rats were separated into four sets.

I-A (normal saline controls). Twenty rats received injections of normal saline.

I-B (left nephrectomy rat serum). Twenty rats received pooled serum from rats submitted to left nephrectomy 48 hr previously. The donor rats were operated upon under ether anesthesia and sacrificed by withdrawing blood from the abdominal aorta. The sera were pooled and stored at −4°. The donor animals showed evidence of right kidney hypertrophy which exhibited 20–30% increase in weight when compared with the weight of the previously removed kidney.

I-C (bilateral nephrectomy rat serum). Twenty rats received pooled sera from bilaterally nephrectomized rats obtained 36 hr after surgery. As in group I-B, the donor rats were operated upon under anesthesia, and blood was drawn from the abdominal aorta. The urea content of this was 117 mg/100 ml.

I-D (sham operation rat serum controls). Twenty rats received serum from rats that had undergone sham operations. Equal amounts of pooled sera from rats undergoing sham operation for left or bilateral nephrectomy were used.

Group II (dog donors). Sixty recipient rats were separated into six sets.

II-A. Ten rats received normal saline.

II-B. Ten rats received canine serum obtained from a dog 48 hr after unilateral nephrectomy.

II-C. Ten rats received serum from the same dog, obtained 7 days after unilateral nephrectomy.

II-D. Ten rats received serum from the same dog, obtained 30 days after unilateral nephrectomy.

II-E. Ten rats received serum from the same dog, obtained 60 days after unilateral nephrectomy.

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II-F. Ten rats received serum from a dog, obtained 48 hr after bilateral nephrectomy. The urea level of the pool was 78 mg/100 ml.

Group III (human donors). Sixty recipient rats were divided into six sets.

III-A. Ten rats received normal saline.

III-B. Ten rats received human serum obtained from a patient 48 hr after unilateral nephrectomy for renal vascular hypertension.

III-C. Ten rats received human serum obtained 7 days after unilateral nephrectomy from the same patient.

III-D. Ten rats received serum from a man with chronic renal insufficiency, and a serum urea level of 280 mg/100 ml.

III-E. Ten rats received serum from another man with chronic renal insufficiency before bilateral nephrectomy. The patient was being treated twice weekly by hemodialysis. The serum urea was 86 mg/100 ml at the time of the experiment.

III-F. Ten rats received serum from the same patient, 48 hr after bilateral nephrectomy. The serum urea was 110 mg/100 ml.

Recipient rats were maintained on Rockland diet throughout the experiment. The recipients were weighed before and after the experiment. Several animals that failed to gain weight during the experiment were not analyzed, but were replaced by fresh rats. This was done to avoid the influence of dietary variations upon the mitotic activity of the kidney (9). Body weight did not vary significantly among the different recipient sets at the time of sacrifice.

Each recipient rat received three injections of 1 ml of serum or normal saline during the experiment, given intraperitoneally at 10-hr intervals. Ten hours after the last injection, each rat received 20 μCi of tritiated thymidine intravenously. One hour later the rats were anesthetized with ether, and both kidneys were removed. All animals were sacrificed in the early afternoon. The whole kidney content of tritium was determined by a modification of the method of Kelly and associates (10), as described by Kurtides and associates for splenic tissue (11). The kidneys were weighed, dried in an oven, and pulverized. One aliquot was weighed and burned in a 2.0-liter heavy-walled flask containing an oxygen atmosphere. The tritium was concentrated by partial immersion of the flask in a Dry Ice–isopropanol mixture. The frozen condensate was subsequently dissolved in 20 ml of toluene–phosphor solution 4.0 g 2,5 diphenyloxazole and 50 mg 1,4-bis (5-phenyl-2 oxazolyl) benzene per liter of toluene; and a 15-ml aliquot was counted in a liquid scintillation spectrometer.

Results. The incorporation of tritiated thymidine by the kidney of groups of 20 normal rats receiving pooled serum from uni- or bilaterally nephrectomized rats was significantly higher than in those receiving normal saline or pooled serum from sham-operated rats. Kidney weights were not significantly different. This was not unexpected, for the rats were sacrified only 31 hr after the initial injection of serum. The data are summarized in Table I.

TABLE I. Tritiated Thymidine (TT) Uptake by Rat Kidney After Administration of Sera from Uni- and Bilaterally Nephrectomized Rats.

	Set	Number of recipients	Both kidneys wet weight (g, $\pm \sigma$)	p value	Renal (TT) uptake (%/g, $\pm \sigma$)	p value
I-A	Normal saline	20	1.71 ± 0.13		0.85 ± 0.06	
I-B	Serum from unilaterally nephrectomized rats	20	1.85 ± 0.31	$>.05$	1.00 ± 0.07	$<.01$
I-C	Serum from bilaterally nephrectomized rats	20	1.83 ± 0.24	$>.05$	0.97 ± 0.03	$<.01$
I-D	Serum from sham-operated rats	20	1.77 ± 0.18	$>.05$	0.88 ± 0.04	$<.05$

TABLE II. Tritiated Thymidine Uptake by Rat Kidney After Treatment with Serum from Uni- and Bilaterally Nephrectomized Dogs.

Set	Number of recipients	Both kidneys wet weight (g, $\pm \sigma$)	p value	Renal uptake (%/g, $\pm \sigma$)	p value
II-A Normal saline	10	1.65 $\pm$ 0.19		0.84 $\pm$ 0.04	
II-B Serum from dog 48 hr after unilateral nephrectomy	10	1.68 $\pm$ 0.16	>.05	0.86 $\pm$ 0.02	>.05
II-C Serum from dog 7 days after unilateral nephrectomy	10	1.67 $\pm$ 0.11	>.05	0.87 $\pm$ 0.07	>.05
II-D Serum from dog 30 days after unilateral nephrectomy	10	1.70 $\pm$ 0.09	>.05	0.83 $\pm$ 0.08	>.05
II-E Serum from dog 60 days after unilateral nephrectomy	10	1.72 $\pm$ 0.18	>.05	0.80 $\pm$ 0.09	>.05
II-F Serum from dog 48 hr after bilateral nephrectomy	10	1.70 $\pm$ 0.20	>.05	0.82 $\pm$ 0.08	>.05

TABLE III. Tritiated Thymidine Uptake by the Rat Kidney After Treatment with Serum from a Unilaterally Nephrectomized Patient and Patients with Renal Insufficiency.

Set	Number of recipients	Both kidneys wet weight (g, $\pm \sigma$)	p value	Renal uptake (%/g, $\pm \sigma$)	p value
III-A Normal saline	10	1.70 $\pm$ 0.14		0.83 $\pm$ 0.06	
III-B Serum from man 48 hr after unilateral nephrectomy	10	1.61 $\pm$ 0.20	>.05	0.81 $\pm$ 0.09	>.05
III-C Serum from man 7 days after unilateral nephrectomy	10	1.75 $\pm$ 0.20	>.05	0.82 $\pm$ 0.07	>.05
III-D Serum from man with chronic renal insufficiency	10	1.65 $\pm$ 0.22	>.05	0.85 $\pm$ 0.07	>.05
III-E Serum from man with chronic renal insufficiency before bilateral nephrectomy	10	1.70 $\pm$ 0.31	>.05	0.86 $\pm$ 0.08	>.05
III-F Serum from man 40 hr after bilateral nephrectomy	10	1.67 $\pm$ 0.17	>.05	0.81 $\pm$ 0.07	>.05

The injection of pooled serum from a dog 2, 7, 30, and 60 days after unilateral nephrectomy did not alter the weight of, nor incorporation of thymidine by the kidney of groups of 10 recipient rats when compared with those receiving normal saline. Likewise, serum obtained from a dog 2 days after bilateral nephrectomy had no effect. These data are summarized in Table II.

The injection of serum obtained from a patient with renovascular hypertension 2 and 7 days after unilateral nephrectomy, from one similar patient with advanced renal insufficiency, and another before and after hemodialysis changed neither the incorporation of tritiated thymidine nor the weight of the kidneys of normal rats when compared with those receiving normal saline. Again, each recipient group consisted of 10 rats.

Discussion. Our results confirm the existence of a factor in the serum of rats after unilateral nephrectomy which affects the growth of kidneys in normal rats. These studies also show that serum from bilaterally nephrectomized rats is equally capable of producing an increase in the incorporation of tritiated thymidine by the kidneys of normal rats. We conclude, therefore, that the factor

is of extrarenal origin.

Several substances have been shown to induce renal hypertrophy, including urea (12), thyroxine, DOCA, progesterone, and testosterone (13). Unilateral nephrectomy in mice is followed by increase in hypophyseal and adrenal weights (14). Thus, hyperfunction of these organs, producing higher excretion of their hormones, might play a role in renal hypertrophy, However, renal hypertrophy after unilateral nephrectomy has been observed in animals subjected to prior thyroidectomy (15), adrenalectomy (16), gonadectomy (17), and hypophysectomy (18). Also, the injection of rat serum obtained after unilateral nephrectomy did not increase the incorporation of tritiated thymidine in the liver (2), evidence against a substance with a general growth-promoting effect.

Subsequent, as yet unpublished data (19), have demonstrated that rat cortical slices incorporate increased amounts of tritiated thymidine into DNA but not RNA after exposure to serum obtained from rats 48 hr after uninephrectomy as compared to serum from sham-operated rats.

Our attempts to demonstrate such a renal hypertrophy factor in the serum of appropriately manipulated dogs or man failed. The idea of species-specificity for the serum renal hypertrophy factor already has been suggested (1). However, this is not unequivocally established. The rat assay animal is so small in relation to dog or human donors that injection of a small standard dose of serum might be insufficient to achieve the effect. Nevertheless, we are inclined to discount this reservation.

Summary. Serum from uni- or bilaterally nephrectomized rats. when injected into normal rats, produced increased thymidine incorporation by their kidneys. Serum from uni- and bilaterally nephrectomized dogs, unilaterally nephrectomized man, and patients with advanced renal insufficiency had no effects on rat kidneys. The findings suggest the existence of a species-specific factor in the serum of nephrectomized rats which stimulates renal hypertrophy.

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