Studies on Compensatory Renal Hypertrophy. I. Effect of Unilateral Ureteral Ligation and Transection.* (30488)

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The removal of one kidney consistently results in compensatory hypertrophy and hyperplasia of the remaining kidney. Although the homeostatic nature of this response is evident, the control mechanisms responsible for compensatory hypertrophy are not well understood. Available evidence indicates that a denervated kidney hypertrophies in response to removal of its partner(1) and hence suggests that humoral agents have a role in regulating kidney growth. Studies(2) reporting the occurrence of a specific kidney growth stimulating factor in the plasma of unilaterally nephrectomized rats have added further support to this possibility.

Earlier attempts to identify a humoral agent or agents responsible for compensatory hypertrophy have assumed that total excretory load was the effective stimulus for regulating kidney growth. For this reason, the effects of feeding and injection of various urinary metabolites have been extensively studied (for review, see(3)). Although it has been shown that increasing the dietary protein can increase total renal tissue mass, there is little evidence to show that products of protein metabolism are responsible for regulating kidney size(4). Furthermore, studies of the effect of increased water and electrolyte load on renal growth have been largely negative(5). These findings suggest that some factor or factors other than alterations in the excretory load are the effective stimulus for producing compensatory renal hypertrophy.

Unilateral ureteral transection or ligation increases the excretory load on the kidney on the unoperated side without a concomitant decrease in total renal tissue mass. Simpson (6), using this approach, has reported on P^{32} incorporation into kidney DNA nucleotides 48 hours after unilateral peritoneal ureterostomy or unilateral nephrectomy. A normal rate of P^{32} incorporation was observed in the unoperated kidney after diversion of the ureter into the peritoneal cavity. In contrast, there was an increased incorporation in the remaining kidney of unilaterally nephrectomized animals. Block *et al* have also reported(7), based on a limited series of animals, that compensatory hypertrophy fails to occur following ureteroduodenostomy.

The observations reported here present evidence, based on kidney weight, that compensatory hypertrophy is retarded by peritoneal ureterostomy. In addition, compensatory hypertrophy of the unoperated kidney was markedly slowed following unilateral ureteral ligation. The interpretation of these findings implies the existence of a controlling factor other than excretory load as one of the parameters responsible for stimulating compensatory renal hypertrophy.

Materials and methods. Albino rats of the Holtzman and Sherman strains were allowed free access to food and water. They were arranged systematically in cages in order to avoid confounding cage and treatment effects. The rats were assigned to experimental groups at random and the groups were balanced to approximately equal initial body weights. All animals received injection of 10,000 units of penicillin and 12.5 mg of dihydrostreptomycin at the time of operation. The surgical procedure was performed through a flank incision. The kidneys were visualized and the ureters ligated or transected, or the kidneys were removed, according to experimental plan. All control animals were sham operated. Although no consistent kidney asymmetry was observed, half of the animals in the control and experimental groups were operated on the left side and half on the right side. Fresh weights following decapsulation and transection of the kidneys (to exclude the weight of urine col-

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TABLE I. Summary of Mean Body Weights and Fresh Kidney Weights.

Exp	Duration in days	Control* N	Control* BW†	Control* KW‡	Transection (control) N	Transection (control) BW	Transection (control) KW	Ligation (control) N	Ligation (control) BW	Ligation (control) KW	Nephrectomy (control) N	Nephrectomy (control) BW	Nephrectomy (control) KW
A	15	10	203	663	10	186	727	10	197	824	10	201	1036
B	11	10	270	983	9	260	1139	9	264	1157	10	280	1325
	21	11	329	1089	10	312	1308	7	302	1372	11	313	1396
C	7	12	168	700	14	155	689	—	—	—	16	173	964
	14	12	198	796	14	190	893	—	—	—	14	209	1184
D	7	10	187	727	12	189	788	10	183	834	11	184	924
	14	11	200	822	11	206	872	10	193	882	12	202	977
	7	11	160	782	—	—	—	11	146	886	11	153	1141
F	26	20	323	1137	—	—	—	16	314	1506	20	313	1597
G	3	14	240	934	12	219	966	—	—	—	12	236	1170
	14	12	303	1082	12	282	1225	—	—	—	11	293	1391
	53	12	380	1192	12	386	1718	—	—	—	12	380	1740
	83	11	421	1244	8	416	1822	—	—	—	16	416	1814

* (Left + right kidney) ÷ 2. † BW = Body wt in g. ‡ KW = Kidney wt in mg.

lected in the pelvis and major calyces) were measured at the time of autopsy. Dry kidney weights were obtained by drying to a constant weight at approximately 68°C. Statistical treatment of the data employed methods described by Snedecor(8).

Results. The kidney weights and body weights shown in Table I are the results of 7 experiments (A through G) performed at various times and of differing durations. The final control kidney weights represent the average of the left and right kidneys of the sham operated control animals. Transection refers to the kidney weight on the unoperated side of animals whose ureter was transected and allowed to drain into the peritoneal cavity. Similarly, ligation refers to the control kidney weight of animals with ligated ureters. Nephrectomy designates the weight of the remaining kidney subsequent to unilateral nephrectomy.

It is apparent in all experiments with the exception of the animals sacrificed at 83 days in experiment G, that the control kidneys of the transected and ligated animals were intermediate in weight between those of the controls and unilateral nephrectomies. To examine these findings more closely, it was necessary to adjust the observed mean kidney weight for the difference in body weight in the several experiments. A linear relationship between the common logarithm of body weight and the mean kidney weight of control animals was observed. An F test showed a highly significant contribution of the regression term to the total variability of kidney weights ($p<.001$). It is important to note that there was no significant effect of nephrectomy on body weight.

The observation of a linear log body weight × kidney weight relationship and the similarity of the final body weight of control and nephrectomized animals permitted the mean final kidney weights in the experimental groups to be adjusted to a constant body weight. This procedure makes it possible to compare directly different experiments so that changes occurring with time can be observed. The results of such a comparison are presented in Fig. 1. All values are shown relative to the hypertrophy that occurred in the unilaterally nephrectomized animals. Several experimental periods of Table I were combined for graphic presentation. The data demonstrate that there was a gradual increase in the control kidney weight of the transected and ligated animals. The hypertrophy reached a maximum value approximately equal to that of the nephrectomized controls by 24 and 83 days respectively in the ligated and transected animals. Statistical comparisons were based on the means of all control and experimental groups. The control kidney weights of the transected and ligated animals were significantly smaller than the mean kidney weights of the nephrectomized animals

TABLE II. Renal Water Content and Standard Errors in mg/g Dry Weight.

Exp duration in days	N	Control*	N	Transection Operated	N	Ligation Operated	N	Nephrectomy Unoperated
7	10	386 (3.7) 0%†	12	473 (13.6) +23%	10	651 (10.4) +69%	11	382 (2.7) −1%
11	10	350 (7.3) 0%	9	518 (28.9) +48%	9	592 (26.3) +69%	10	342 (8.2) −2%
21	10	318 (2.6) 0%	10	507 (19.2) +59%	7	548 (10.2) +72%	10	333 (3.7) +5%

* (Left + right kidney) ÷ 2. † % Change from control.

($p<.001$ and $p<.025$) and heavier ($p<.01$) than the kidney weight of the control animals.

The water content in milligrams per gram dry weight for experiments B and D are shown in Table II. In contrast with Table I, the water content of the kidneys from the *operated* side of the transected and ligated animals is presented. It is apparent from these data that only minor variations occurred in the kidneys of control and unilaterally nephrectomized animals. The kidneys from the operated side of the ligated animals, however, showed a marked increase in water content. Seven days after ureteral ligation there was a 69% increase in water content over the control kidneys. Ureteral transection also produced hydronephrosis but in this case it developed more slowly. Tests of significance indicated that at all experimental durations—7, 11 and 21 days—there was a significant ($p<.01$) increase in water content of the transected and ligated kidneys over that of the controls. The difference between the operated groups was significant ($p<.01$) at 7 days but not at 11 and 21 days. The appearance of the kidneys at time of autopsy confirmed that hydronephrosis occurred on the operated side of both ligated and transected animals, and that it was more severe in the ligated groups.

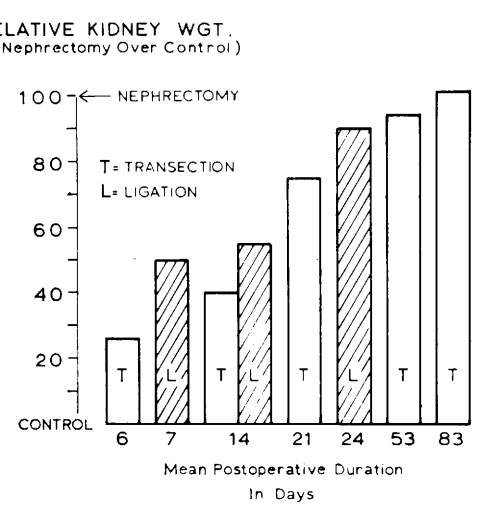

FIG. 1. Relative compensatory hypertrophy of control kidneys following transection or ligation of contralateral ureter. (Expressed as % of nephrectomy over intact control.)

It was noted previously that compensatory hypertrophy occurred in the control kidney of animals whose contralateral ureter had been transected for 83 days. The results presented in Table III are of interest in this connection. The dry kidney weights of both the control and operated side of transected animals are compared with the kidneys of control and unilaterally nephrectomized animals. There was a gradual increase in weight of the control kidney of the transected animals that by 83 days had approximately equaled that of the hypertrophied kidney of the unilaterally nephrectomized animals. However, the hypertrophy was significantly less ($p<.001$) than occurred in nephrectomized animals at 3 and 14 days. The increased weight of the control side at 53 and 83 days was accompanied by a striking and significant decrease in the dry weight of the operated side relative to that of the control animals.

Discussion. The factors responsible for the regulation of kidney growth are complex and not well understood(3). It is reasonable to assume, however, that changes in kidney size observed following removal of one kidney, subserve the functional needs of the organism. Our findings indicate that the most effective stimulus for production of compensatory hypertrophy and hyperplasia is related

TABLE III. Mean Dry Kidney Weights* and Standard Errors in mg, Exp G.

Exp duration in days	Control† N		Transections N	Control	Operated	Nephrectomy N	Control
3	13	204 ± 10.6 0%‡	10	216 ± 10.6 +35%	214 ± 10.6 +28%	10	241 ± 10.6 +100%
14	11	243 ± 18.4 0%	11	306 ± 18.4 +70%	270 ± 18.4 +30%	10	333 ± 18.5 +100%
53	12	293 ± 27.1 0%	11	402 ± 27.1 +89%	163 ± 27.1 −107%	12	415 ± 27.0 +100%
83	11	297 ± 30.9 0%	8	430 ± 31.4 +111%	124 ± 31.4 −145%	16	417 ± 30.5 +100%

* Adjusted for body wt differences at each period. † (Left + right kidney wt) ÷ 2.
‡ % Change from control.

in some manner to a reduction in total renal tissue mass. This conclusion appears warranted from the result showing that hypertrophy is significantly slower if, instead of removing one kidney, its ureter is either ligated or diverted to drain into the peritoneal cavity.

The hypertrophy which occurs in the control kidney following ureteral transection or ligation can be explained at least in part by the hydronephrosis and the resulting progressive destruction of renal tissue on the operated side. This interpretation is supported by the observation that a greater degree of hypertrophy occurs in the control side of the ligated animals than in those whose ureters were transected. The data on water content (Table II) confirm that the ligated animals experienced the greater and more rapid distortion of renal structure. The results shown in Table III indicate that compensatory hypertrophy of the control kidney following ureteral transection was closely related to the destruction of renal tissue on the operated side. It should be noted that the failure of the operated side to show a decrease in dry kidney weight at 14 days probably reflected the increase in size of the distended renal pelvis and calyces. Although it is attractive to ascribe all hypertrophy to the loss of renal tissue as a result of hydronephrosis this would seem premature pending exact measurement of the amount of functional tissue remaining on the operated side of the transected and ligated animals.

The observation that the kidney regulates the ionic composition of the extracellular fluid primarily by reabsorbing components of the glomerular filtrate suggests that "reabsorptive load" rather than "excretory load" may be the critical factor responsible for compensatory hypertrophy. This supposition is strengthened by the finding that the energy requirements for sodium reabsorption account for the major proportion of the oxidative metabolism of the kidney(9). This suggestion fits well with our finding that compensatory hypertrophy is slowed following ureteral ligation of peritoneal ureterostomy since in both circumstances the reabsorptive load of sodium would be expected to remain relatively constant on the unoperated side. That this is not a complete explanation, however, is evident from a consideration of the results of unilateral nephrectomy, since in this case the filtered sodium load to the remaining kidney would be unaltered unless glomerular filtration rate is markedly increased. Unpublished function studies in dogs suggest that an increased G.F.R. may indeed occur. The problems, however, of the time relations between the functional and morphological changes remain open. The nature of the effective stimulus producing an elevated G.F.R. in response to a decrease in renal tissue mass is also unknown.

Summary. Data are presented comparing the relative rate of compensatory renal hypertrophy and hyperplasia of the control kidney of rats following either unilateral ureteral transection, ligation, or nephrectomy. The growth of the control kidney on the unoperated side was strikingly slower after ureteral transection and ligation when compared with that resulting from the removal of one kidney. Progressive hydronephrosis was observed

on the operated side following ureteral transection and ligation and was more pronounced in the latter. Degree of hypertrophy of the control kidney showed a close correlation with degree and duration of the hydronephrosis of the kidney on the operated side. These observations have been interpreted as indicating the operation of a control system regulating kidney growth in response to changes in some way related to kidney size.

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Infusion of Sodium Bicarbonate-C^{14} in Sheep.* (30489)

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The oxidative metabolism of various substrates in ruminants has been studied by single injection(1,2,3) and constant infusion techniques(4,5). These techniques usually require interpretation of expired carbon dioxide data to estimate the oxidative rate. Although the time rate of oxidation is not actually measured, it is assumed to be proportional to the rate of elimination of carbon dioxide in expired air. Existence of large carbon dioxide pools in the body with which metabolically produced carbon dioxide mixes to a variable extent before it is expired, could lead to erroneous oxidative rates. The advantages of continuous infusion over single injection methods has been discussed by Morris and Simpson-Morgan(6).

The purpose of this study was to study the carbon dioxide pool system of sheep using constant infusion of sodium bicarbonate-C^{14}.

Methods. Two yearling grade wethers were used for a total of 3 infusions, each 4 hours in duration. A recovery period of about 4 months was allowed between experiments when the same sheep was used for 2 infusions. Sodium bicarbonate-C^{14}, dissolved in physiological saline, was infused by means of an automatic infusion withdrawal pump through a polyethylene catheter (I.D. 0.062″, O.D. 0.082″) inserted into the right jugular vein. Blood samples were withdrawn through an indwelling catheter inserted into the left jugular vein. The catheters were threaded into the veins through an 11-gauge hypodermic needle the day before infusions were conducted.

The amount of acid-releasable CO_2 in whole heparinized blood was determined manometrically as described by Gupta *et al* (7). Hydrochloric acid with a normality of 0.5 was used to release CO_2 rather than 0.2% citric acid used by Gupta *et al.* For radioactivity assay, acid-releasable CO_2 was collected in 2 N sodium hydroxide and an aliquot removed and added to a scintillator solution described by Harlan(8), composed of Cab-O-Sil, ethanol, toluene, 2,5-diphenyloxazole (PPO), and 5-phenol-oxazole (POPOP), and counted in a Packard Tri-Carb Scintillation Spectrometer.

Rumen fluid was obtained through a ru-

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