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Compensatory Growth in the Rat Kidney: Effect on Collagen Mass (40187)

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It is well known that following unilateral nephrectomy in mammals, the remaining kidney undergoes a marked compensatory increase in both weight and function (1, 2). During this process of compensatory growth, both the size and number of renal cells increases, though the number of nephrons remains unchanged (3, 4). Protein content of hypertrophied kidneys has been shown to increase with a corresponding increase in RNA and a small increase in DNA synthesis (4–6). The process of protein biosynthesis and the kind of proteins that accumulate during compensatory renal hypertrophy have not been studied in detail.

The collagen content of rat kidneys, measured as hydroxyproline content, ranges from 1.8 to 3.3 percent of dry kidney weight (7, 8). Renal collagenous tissues are mainly basement membranes and interstitial connective tissue. Little is known about the changes in renal collagen during kidney compensatory growth. From the few previous studies, it seems that total collagen is being accumulated to a greater extent in hypertrophy than in controls (9, 10). Karen *et al.* (10) have found the renal concentration of collagen to increase, but Montfort and Perez-Tamayo (11) observed the same parenchyma/collagen ratio in normal and hypertrophic kidneys. In those studies (10, 11), the information is based on observations from one or mostly two points in time.

The aim of the present study was to determine quantitatively the changes in the total amount of collagen per kidney, as well as renal collagen concentration during compensatory renal growth in rats. Observations were made from 10 to 120 days after unilateral nephrectomy and correlated with the degree of kidney hypertrophy.

Materials and methods. Twelve-week-old male Lewis rats weighing 250 ± 20 g (mean ± SD) were used for these experiments. The animals were divided into three groups. Group I consisted of 10 rats which were sacrificed at the beginning of the experiments to determine the variation of renal mass and collagen in the right and left kidneys. Both kidneys were removed and compared for wet weight, dry, fat-free weight, collagen content per whole kidney and collagen concentration per dry weight. Group I served as control for the other groups. Group II consisted of 25 rats which underwent unilateral nephrectomy. The operative procedures were performed under ether anesthesia. The left kidney was approached through a midline abdominal incision, the renal vascular pedicle was ligated and the kidney was removed. Group III consisted of 25 rats which served as a control of normal kidney growth for comparing to the unilateral nephrectomy group.

All the animals were allowed free access to water and fed Purina laboratory chow *ad libitum.* Growth rates were comparable in all groups as measured by a similar gain in body weight.

Subgroups of five rats each from the unilateral nephrectomy group and an equivalent number of rats from the control group were sacrificed at 10, 20, 30, 60, and 120 days after the nephrectomy. The kidneys were carefully dissected free of surrounding tissue and removed. After blotting and removing the perinephric fat, the kidneys were weighed and stored at −70° centigrade until used for later analysis. At the end of the experiment, all the kidneys were cut into small pieces and chloroform–methanol mixture (2:1) was added to the fragmented kidney for the removal of lipids. After 18 hr, the chloroform–methanol was decanted and the entire procedure was repeated three times. The delipidated kidneys were dried at room temperature for three to 4 days in a desiccator at a pressure of 1 mm of mercury over sodium hydroxide pellets. The dried kidneys were hydrated in 6 *N* HCl acid for 30 min on a steam table and then

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hydrolyzed for 3 hr. The hydrolyzates were analyzed for hydroxyproline by a modification (12) of the method of Neuman and Logan (7). The amount of hydroxyproline per sample was converted to collagen by the factor 7.46 (7). Collagen "content" of the kidney was defined as mg collagen/whole kidney and collagen "concentration" as mg collagen/100 mg dried, fat-free weight of kidney.

Results. All the rats gained weight during the experiment. At 0 time, body weight of Group I was 242 ± 13 g (mean ± SD), Group II; 244 ± 13 g and Group III; 241 ± 12 g, respectively. The mean body weights of corresponding control and experimental groups were similar for each time interval. The body weights increased progressively over the 120 days studied when they reached the weight of 418 ± 29 g.

The mean wet weights of right and left kidneys from Group I was 1068 ± 115 mg. In most instances, the right kidney was heavier with the mean percent difference of right over left being 5.0%. The average dry, fat-free kidney weight was 238 ± 25 mg, the right side weighed 3.7% more. The amount of collagen per whole kidney was almost identical, 5.43 ± 0.32 mg in the right kidney compared to 5.40 ± 0.31 mg in the left kidney. Mean collagen concentration of the right kidney was 2.24 ± 0.22 mg/100 mg dry weight and was 3.5% greater for the left kidney (Table I). These variations in kidney weight and collagen are statistically insignificant (P value > 0.2). Therefore, the side-to-side difference has little influence on the response of the remaining kidney mass, as the masses of both kidneys are comparable.

The absolute and relative changes in kidney dry fat-free weight, collagen content and collagen concentration are demonstrated in Figs. 1–3. During the first 20 days after nephrectomy the remaining kidneys were gaining weight at a higher rate than in the next 100 days (Fig. 1). The relative increase in the weight of the hypertrophic kidneys was 153% of the control at 20 days, reaching 160% at 120 days, (Fig. 2). The total dry weight for experimental and control groups was smaller at 120 days in comparison to 60 days (Fig. 1).

In contrast to the rapid increase in dry weight, the amount of total collagen per kidney increased slowly at the beginning (Fig. 3). An increase, 118% of the control, (from 6.21 to 7.35 mg) occurred by 20 days and reached 156% (from 7.88 to 12.30 mg) by 120 days (Fig. 2). At this time, the percentage increase in the kidney collagen was similar to the percentage increase in dry weight (156% and 160% respectively).

When comparing the mass of one hypertrophied kidney to the total mass of the right and left control kidneys at 10 days, the hypertrophied kidney weighed 68% of the control kidneys and reached 80% by 120 days. A similar comparison for the accumulation of

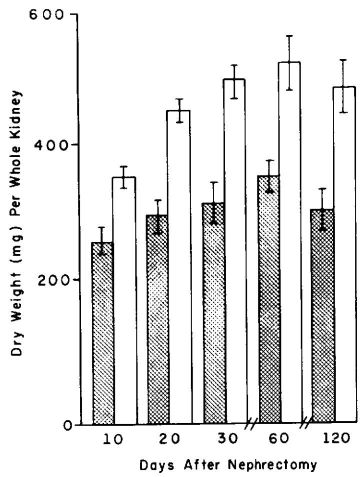

FIG. 1. Dry, fat-free weight (mg whole kidney), 10–120 days after unilateral nephrectomy. Dashed bars—control; open bars—compensatory growth. Data are presented as mean ± SD when n = 5 and the differences are significant at a P values <0.001.

TABLE I. VARIATION IN KIDNEY WEIGHT AND COLLAGEN IN CONTROL RATS.

	Wet weight (mg)	Dry, fat-free weight (mg)	Collagen content (mg/kidney)	Collagen concentration (mg/100 mg dry weight)
Left kidney	1044 ± 109	233 ± 24	5.40 ± 0.31	2.32 ± 0.18
Right kidney	1092 ± 121	242 ± 26	5.43 ± 0.32	2.24 ± 0.22

[a] Data are presented as mean ± SD when n = 10, bw 242 ± 13 g.
[b] Collagen is obtained by multiplying the amount of hydroxyproline by 7.46 (7).

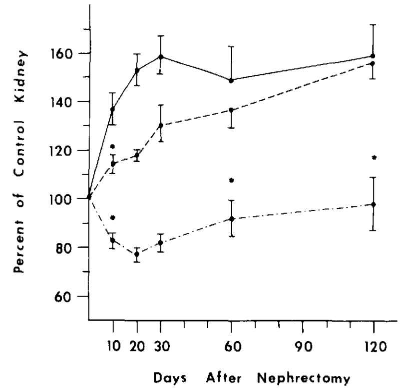

FIG. 2. The rat kidney's data for fat-free, weight (——), collagen content (- - -) and collagen concentration per dry kidney weight (-·-·-·) are presented as percent of control data. The vertical bar represents the standard deviation. Values after uninephrectomy were different at the $P < 0.001$ level at each time except where asterisks are given ($P < 0.05$).

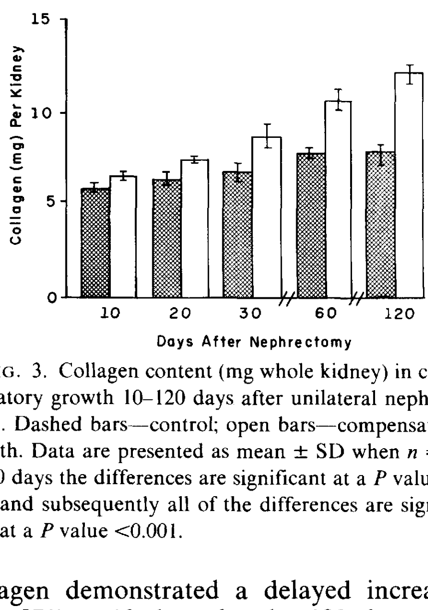

FIG. 3. Collagen content (mg whole kidney) in compensatory growth 10–120 days after unilateral nephrectomy. Dashed bars—control; open bars—compensatory growth. Data are presented as mean ± SD when $n = 5$. At 10 days the differences are significant at a P value < 0.05 and subsequently all of the differences are significant at a P value <0.001.

collagen demonstrated a delayed increase, only 57% at 10 days, but by 120 days, the hypertrophied kidney reached 78% of the total collagen of both kidneys.

The concentration of collagen in the control was 2.28 ± 0.21 mg/100 mg dry weight and did not change significantly until 120 days when it was 2.70 ± 0.18 mg/100 mg. In the experimental groups, the collagen concentration reached a minimal value of 1.66 ± 0.06 mg/100 mg dry weight (77% of the control, Fig. 2) at 20 days and thereafter increased to a value of 2.28 ± 0.15 mg/100 mg at 60 days which was similar to the control.

Discussion. The difference in kidney weight, collagen content and collagen concentration of the right kidney compared to the left was found to be insignificant. These right to left comparisons are similar to the reported observations in other strains of rats (1, 4, 15). It supports the validity of the assumption that the hypertrophic kidney (right side in our experiment) can be compared to the control kidneys without being affected by side-to-side variation.

In regard to the possible influence of surgical insult (like a sham operation) on kidney weight and collagen, we have found no significant change after inducing renal ischemia by occluding the renal artery for 20 minutes (unpublished data). This is consistent with kidney sham operations (18–20) which showed no significant increase in renal DNA and RNA. In reports indicating an increase in renal DNA and RNA after sham uninephrectomy, the sham operations were complicated by manipulation of the kidney. The kidneys were either touched with instruments wet with isopropanol (18) or pararenal fat was removed which may result in surface damage of the kidney (21).

Some investigators have used wet weight of the kidney as a measure of hypertrophy (1, 13). This method is insensitive as it can be affected by such factors as hydration state of the animal or blood and urine content of the kidney. The dry, fat-free kidney mass represents mainly total kidney proteins, therefore, changes in protein are reflected in the dry weight of the kidney (14). The increase in the dry weight of the hypertrophic kidney was almost completed by 20 days after unilateral nephrectomy and from that point until 120 days there was only a small additional increase. The dry weight of the hypertrophic kidney reached 80% of the combined weight of the control right and left kidney masses by 120 days. Thus, it seems that our compensatory increase approaches the maximal degree of compensatory growth for this age group. These observations are comparable to previous studies that had an increase of kidney mass and which were completed within 5 to 6 weeks (13, 14, 16).

Our data demonstrated that renal collagen mass increases continuously during compensatory growth. Initially, the increase in collagen mass is not as rapid as the increase in renal dry mass, but the increase continues after the major changes in dry weight have stabilized. It is difficult to correlate the degree of the increase in collagen mass observed here with the few previous reports (9–11) since their figures on collagen were given only as collagen concentration per dry weight.

In contrast to the previously reported increase in collagen concentration in hypertrophic kidneys at 28 days (9) or no change from normal kidneys at 10 and 20 days (11); we have observed a significant decrease in collagen concentration at early stages after contralateral nephrectomy followed by a subsequent increase. This difference may be caused by the earlier increase in other components of the dry kidney weight at this period (mainly other proteins). At later stages, the increase in collagen mass exceeds the increase of renal dry mass and is expressed by an increased concentration of collagen (Fig. 2).

This increase of renal collagen may occur in the basement membrane of glomeruli and tubules (type IV collagen) and/or interstitial connective tissue (mainly type I collagen with some type III collagen). The chemical analysis used for collagen determination does not distinguish the various types of collagens, however, other studies (9, 17) suggest that most of the increase may be in the basement membrane. A microscopic study (17) of compensatory hypertrophic kidneys demonstrates thickening of the glomerular basement membrane, and disc gel electrophoresis of whole renal collagen (9) indicated an increase in basement membrane collagen.

Summary. The net changes in collagen mass and collagen concentration of kidneys, measured by hydroxyproline, were determined after unilateral nephrectomy in 12-week-old Lewis rats. Observations were done 10 to 120 days after nephrectomy. Compensatory growth shows a distinct increase in collagen mass of the remaining kidney. By 120 days, the renal dry weight in the hypertrophic kidney increased to 160% of the control. At that time, the collagen mass per whole kidney increased to 156%. While the increase in dry weight is almost completed by 20 days, the collagen mass continued to increase during the 120 days of the study.

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