Influence of Age on the Compensatory Response in Growth and Function to Unilateral Nephrectomy

JOHN H. GALLA, TRUDY KLEIN-ROBBENHAAR,
AND JOHN P. HAYSLETT

Departments of Medicine and Pediatrics,
Yale University School of Medicine, New Haven, Connecticut 06510

Received June 11, 1974

The effect of age on the structural and functional response to unilateral nephrectomy was studied in weanling (group I) and young adult (group II) rats. Although compensatory growth in group I was not significantly greater than in group II one week following surgery (44% vs 39%, p - NS), after 4 wk renal mass had increased 144% in group I and 66% in group II (p < .0001). Glomerular filtration rate per unit kidney mass at 1 wk post surgery was 875 ± 92 (mean ± SEM) μl/min/gKW) in group I and 1132 ± 67 in group II (p - NS) and at 4 wk was 1176 ± 67 in group I and 1261 ± 67 in group II (p - NS). These data indicate that the magnitude of compensatory growth in immature rats is greater than in adults and that functional adaptation parallels the structural change.

INTRODUCTION

It is well known that following removal of one kidney, the remaining kidney undergoes a rapid and marked increase in both size and function. Although earlier studies have suggested that this adaptive response is age dependent, there is no evidence to show that the change in renal function is greater in young immature animals than in adults. The present study, therefore, was explicitly performed to compare the effect of unilateral nephrectomy in young immature to adult animals. Since both the rate of change as well as the final response was of interest, an analysis of compensatory growth and the functional response to unilateral nephrectomy was made one week after surgery, during the period of rapid adaptation, and after one month when changes were presumably complete.

1 The work was supported by USPHS Grant No. HL AM 13647 and the American Heart Association.
2 Dr. Hayslett is an Established Investigator of the American Heart Association. Dr. Galla was supported by the Connecticut Heart Association. Dr. Galla's present address is the Department of Medicine, University of Kentucky, Lexington, KY 40506.
METHODS

Studies were carried out in two groups of male albino Spargue–Dawley rats (Charles River Breeding Laboratories, Wilmington, Ma). Throughout the study, animals were allowed free access to standard Purina rat pellet diet and tap water. The first group consisted of 29 weanling animals weighing initially between 50 and 80 g and the second group of 32 young adult rats weighing initially between 155 and 210 g. The two age groups were divided into experimental and control groups. Under light ether anesthesia, experimental animals underwent a right nephrectomy through a flank incision. After dissection of perirenal fat and other extrarenal tissue, the kidney was lightly blotted and placed in a tared sealed weighing bottle to determine wet kidney weight (KW). Control animals were subjected to a sham operation. Experimental and control animals were further sub-divided into groups in which clearance studies were conducted at 1 wk and 4 wks after surgery.

For clearance studies, animals were weighed and anesthetized with Inactin (Promonta/Hamburg) in a dose of approximately 100 mg/kg, intraperitoneally. Tracheostomy was performed and animals were placed on a heated board. Rectal temperatures were monitored by a telethermometer (Yellow Springs Instruments Model 43TA) and were maintained between 36.5 and 38.5°C. PR-50 polyethylene catheters were placed into the left external jugular vein and into the bladder through midline incisions. Prior to the administration of priming solutions, NaCl 0.15M, in a volume equal to one percent of body weight (BW), was given intravenously over a 5 min period as a standard replacement of surgical losses. An intravenous priming dose of 11C inulin (New England Nuclear Corp.) was administered rapidly and followed by a sustaining infusion delivered by a constant infusion pump (Harvard Apparatus Co., model 975). The weanling animals received a priming dose of 3.0 μC as 0.06 ml of a 50 μC 11C inulin/ml solution and a sustaining infusion of 7.5 μC 11C inulin in 1.2 ml normal saline/hr. Adult animals received a priming dose of 3.5 μC as 0.08 ml of a 50 μC 11C inulin/ml solution and a maintenance infusion of 10 μC in 1.2 ml normal saline/hr. After a 30 min equilibration period, two consecutive 30 min urine collections were obtained under mineral oil. Tail vein blood samples were collected in heparinized capillary tubes at the beginning and end of each urine collection period, promptly centrifuged and the plasma separated. Animals were then sacrificed and the left kidney was removed to determine wet weight as previously described.

Urine volumes were measured in 100 μl capillary pipets. For the determination of inulin concentration, 20 μl aliquots of plasma and 1 μl aliquots of urine in duplicate were counted in 10 ml Aquasol (New England Nuclear Corp.) in a Packard Tri-Carb liquid scintillation spectrometer, Model 3320. Kidneys were weighed using a Sartorius analytical balance.

Inulin clearance (Ci,n) was calculated from the equation Ci,n = UV/P in which U and P are the urine and plasma concentrations in inulin in counts per minute per microliter and V is urine flow rate in microliters per minute. Plasma inulin was taken as the mean of the initial and final values obtained for each urine collection period.

In order to determine the absolute and relative change in KW from the time of uninephrectomy or sham surgery to the final clearance study, the initial left KW in experimental animals was assumed to be equal to the weight of the excised right
kidney. For control animals, the initial left KW was assumed to be equal to the mean weight of the excised right kidney of the experimental animals—initial experimental and control mean BW being approximately equal in all groups. The validity of this assumption was demonstrated in a previous analysis from our laboratory which showed neither a statistical difference in weight between right and left kidney of the same animal nor a difference in KW between animals of approximately equal BW (12). The absolute change (ΔKW) was estimated as the difference between final and initial KW and relative change (%ΔKW) as that difference divided by initial KW. To estimate the absolute increment in KW due to the adaptive response to uninephrectomy, the observed increase in KW of control animals—due solely to normal growth—was subtracted from the observed increase in experimental animals—due to both normal and compensatory growth:

\[ \Delta KW \text{ (Compensatory)} = \Delta KW \text{ (Exp)} - \Delta KW \text{ (Cont)} \]

—the assumption being that normal growth was similar in both groups. Relative-change was calculated from a similar expression:

\[ \% \Delta KW \text{ (Compensatory)} = \% \Delta KW \text{ (Exp)} - \% \Delta KW \text{ (Cont)} \].

The statistical significance between experimental and control groups was determined using standard statistical methods and Student’s t test (22). For all kidney weights, the F test for homogeneity of variance failed to detect significant differences among the groups (\( F_{max} = 10.53 \)) permitting the use of a pooled estimate of variance (\( Sp (80) = 0.102 \)). This estimate was used in testing the significance of the differences in the compensatory increase in renal mass between weanlings and young adults shown in Table 2.

RESULTS

Change in body weight of control and experimental animals. The rate of compensatory as well as normal renal growth is influenced by caloric and nitrogen intake (8, 14, 18, 19). As shown in Table 1 and Fig. 1, the mean initial, final and change in body weight which reflects caloric and nitrogen intake of experimental groups were nearly identical to control animals. Moreover, from inspection of Fig. 1 it is evident that weight gain of experimental and control animals was similar during the first week, as well as during the 4 wk after surgery.

Influence of age on compensatory renal growth after removal of one kidney. Removal of one kidney resulted in the expected compensatory growth of the remaining kidney in experimental animals as shown in Fig. 2. Though a greater change in compensatory growth was found in animals operated upon during the weanling period compared to adult rats, this difference was observed only after the period of initial rapid growth. Comparison of the weight of the hypertrophied kidney to kidney weight in sham operated control rats demonstrated a 30 and a 35% difference in weanling and adult animals, respectively, 1 wk after surgery. One month after operation, the differences were 51 and 42% in the same groups, respectively.

The striking capacity for compensatory renal growth in young immature rats was evident from inspection of absolute changes in renal mass as shown in Fig. 3A. At the time of surgery the initial kidney weight was 0.38 ± 0.02 g (mean
| TABLE 1                                      |
|---------------------------------------------|
| SUMMARY OF CHANGES IN BODY WEIGHT (BW), KIDNEY WEIGHT (KW) AND RENAL FUNCTION FOLLOWING UNILATERAL NEPHRECTOMY* |

|          | 1 wk post-nephrectomy | 4 wk post-nephrectomy |          |          |          |
|----------|------------------------|------------------------|----------|----------|----------|
|          | Weanling               | Young adult            | Weanling | Young adult |
|          | Units                  |                        |          |          |          |
| No. of animals | 8                      | 7                      | 7        | 7        | 9        |
| Initial BW  | g                      | 66 ± 4                 | 69 ± 4   | 183 ± 3  | 180 ± 6  | 72 ± 4   | 69 ± 3   | 203 ± 6  | 198 ± 3  |
|            | P = NS                 |                         |          |          |          | P = NS   |          | P = NS   |
| Final BW   | g                      | 107 ± 6                | 112 ± 6  | 235 ± 4  | 237 ± 11 | 279 ± 8  | 284 ± 10 | 384 ± 7  | 384 ± 10 |
|            | P = NS                 |                         |          |          |          | P = NS   |          | P = NS   |
| Interval  | g                      | 41 ± 3                 | 43 ± 2   | 52 ± 4   | 58 ± 7   | 207 ± 8  | 216 ± 10 | 180 ± 4  | 183 ± 9  |
| change BW  | P = NS                 |                         |          |          |          |          |          |          |
| Initial KW | g                      | 0.380 ± .020           | 0.380    | 0.828 ± .027 | 0.828 | 0.395 ± .020 | 0.395 | 0.876 ± .034 | 0.876 |
|            | P < 0.005              |                         |          |          |          | P = NS   |          | P = NS   |
| Final KW   | g                      | 0.735 ± .035           | 0.566 ± .025 | 1.253 ± .044 | 0.929 ± .035 | 1.684 ± .043 | 1.115 ± .037 | 1.927 ± .060 | 1.352 ± .025 |
|            | P < 0.005              |                         |          |          |          | P < 0.001 |          | P < 0.001 |
| Interval  | g                      | 0.355 ± .027           | 0.186 ± .025 | 0.425 ± .035 | 0.101 ± .024 | 1.289 ± .043 | 0.720 ± .037 | 1.050 ± .046 | 0.476 ± .025 |
| change KW  | P < 0.001              |                         |          |          |          | P < 0.001 |          | P < 0.001 |          |
| %         | 95 ± 9                 | 49 ± 6                 | 52 ± 4   | 12 ± 3   | 332 ± 20 | 182 ± 9  | 120 ± 7  | 54 ± 3   |
|           | P < 0.002              |                         |          |          |          | P < 0.001 |          | P < 0.001 |          |
| C_{in}/BW  | µl/min/100 g BW        | 665 ± 50               | 964 ± 62  | 604 ± 42 | 806 ± 42 | 708 ± 35 | 983 ± 43 | 629 ± 30 | 761 ± 23 |
|           | P < 0.005              |                         |          |          |          | P < 0.005 |          | P < 0.005 |          |
| C_{in}/KW  | µl/min/g               | 975 ± 92               | 968 ± 64  | 1192 ± 67 | 1024 ± 71 | 1176 ± 67 | 1242 ± 67 | 1261 ± 71 | 1071 ± 35 |
|           | P = NS                 |                         |          |          |          | P = NS   |          | P < 0.05  |

* Values represent mean ± SE. Statistical comparison of each experimental group to its respective control group is indicated.
Fig. 1. The change in body weight 1 and 4 wk after surgery in weanling and adult rats.

Fig. 2. The initial and final kidney weight in weanling and adult rats.

±SE) in weanling rats and 0.83 ± 0.03 g in adults. Although the absolute change in mass due to hypertrophy in the experimental kidney, ΔKW (Compensatory), 1 wk after nephrectomy was greater in adult animals (0.32 ± 0.05 g) than in weanlings (0.17 ± 0.05 g) (P < 0.05), absolute change in mass due to hypertrophy
was equal in both age groups by 4 wk (Fig. 3A and Table 2) and averaged approximately 0.57 g (P not significant).

Comparison of the relative change in renal mass (experimental kidney vs control), %ΔKW (Compensatory), between weanling and adult animals was also made to determine whether compensatory growth was greater in immature animals. As illustrated in Fig. 3B, the hypertrophied kidney grew 44% more than control in weanling rats and 39% in adults during the first week post-operatively. This difference was not significant (Table 2). During the subsequent 3 wk, however, a markedly greater response was found in immature animals. One month after surgery the percent change in renal mass due to compensatory growth was 144% in weanling rats compared to 66% in the adult group (P < 0.0001).

Influence of age on functional adaptation after removal of one kidney. In order to determine whether the functional adaptation after partial ablation of renal mass was greater in immature animals the glomerular filtration rate per unit kidney mass (C₁₈/KW, μl/min/g KW) in immature animals was compared to adult rats. When expressed in this way there was no difference in renal function 1 wk or 4 wk after surgery between immature and adult animals. As shown in Table 1, the C₁₈/KW was 975 ± 92 (mean ±SE) weanling, and 1132 ± 67, adults, 1 wk after surgery (P not significant) and 1176 ± 67, weanling, and 1261 ± 71, adults, 4 wk after operation (P not significant). These data indicate that the functional response occurs pari passu with the change in mass and is therefore greater when surgical ablation is performed in immature animals.

DISCUSSION

Previous studies have shown that after removal of one kidney in adult rats the remaining kidney increases approximately 40% in mass and 60–70% in function (1, 5, 10, 13, 20). This response occurs rapidly following surgery and is complete within 3–4 wk (11, 13). Earlier workers suggested that the capacity for compensatory growth is greater in young, sexually immature animals than in adults, and is probably attenuated with advanced age (3, 4, 6, 9, 10, 15). These reports were
largely based on a comparison of the rates of synthesis of DNA and RNA or the
final kidney weights of uninephrectomized and control animals and did not estimate
functional response. In a recent study Dicker and Shirley (6) estimated renal hyper-
trophy in 5 day old rats and adult animals by comparing the weight of the hypertrophied kidney to that of control sham-operated animals. They found that
from 9 days after the operation the magnitude of renal compensatory hypertrophy
was greater in young immature rats.

In the present study two age groups were examined to correspond to differences
in normal growth, characterized chiefly by hyperplasia in one and by hypertrophy
in the other. From studies of DNA, RNA and total protein content in normal rats
the growth cycle of the kidney has been divided into three phases (23). The first
phase of normal growth extends from birth to about the 14th day of life and is
characterized by similar increases in DNA and protein content, changes interpreted
as indicating rapid cell division of hyperplasia. In the second phase which begins
during the 3rd week and continues until the 40th day renal mass increases because
of hypertrophy and hyperplasia. Thereafter, total DNA content ceases to increase
while protein accretion continues. The third phase which persists during a greater
portion of adult life is, therefore, characterized by hypertrophy. Based upon this
information we can expect that when surgery was performed in the weanling group,
at 3–4 wk of age, normal renal growth was in large part due to hyperplasia. In
contrast, since descent of the testes occurs in the albino rat at approximately 42
days of age and a body weight of 120 g (2, 7), the adult group was studied when
sexually mature and at a time when normal renal growth was primarily due to
hypertrophy. It is important to note that this analysis was performed under condi-
tions in which the caloric, nitrogen and electrolyte intake of control and experi-
mental groups was similar, since these factors have been shown to influence both
normal and compensatory renal growth (8, 14, 18, 19).

It is apparent from the measured rates of renal growth in the remaining kidney
of experimental rats that the compensatory response was markedly greater in young
sexually immature animals than in adult rats. This difference in compensatory re-
response, however, was not found during the initial phase of rapid compensatory

### Table 2

|                      | Mean difference ± 1 S.E. |
|----------------------|-------------------------|
|                      | Weanlings 1 wk | Young adults 1 wk | t       | P        |
| Absolute change      | 0.17           | 0.32              | -2.04   | <.05     |
| ΔKW (Compensatory) g | ±0.05          | ±0.05             | 0.40    | NS       |
| Relative change      | 44.5           | 39.1              | 0.05    | NS       |
| %ΔKW (Compensatory)  | ±15.2          | ±5.9              |         |          |

|                      | Weanlings 4 wk | Young adults 4 wk | t       | P        |
| Absolute change      | 0.57           | 0.58              | -0.08   | NS       |
| ΔKW (Compensatory) g | ±0.05          | ±0.05             |         |          |
| Relative change      | 144.1          | 65.6              | 4.23    | <.0001   |
| %ΔKW (Compensatory)  | ±18.1          | ±4.0              |         |          |

* Each value represents the mean difference ± SE between the experimental and control values for weanling and adult animals at 1 and 4 wk after surgery.
growth but was achieved by continued growth after the first week following surgery. The remarkable response in compensatory growth achieved by the young animal is illustrated by the similar absolute increase in renal mass due to hypertrophy found 1 mo following surgery in both age groups, despite the difference in estimated initial kidney weight. It should be noted that the enhanced rate of compensatory renal growth in weanling rats occurred in association with a greater rate of normal growth. Renal mass in control animals increased 49 and 182% during the subsequent 1 and 4 wk periods in the weanling groups compared to 12 and 54%, respectively, in adult animals.

The demonstration that glomerular filtration rate per unit renal mass was similar in both age groups at 1 and 4 wk after surgery indicates that mass and function change pari passu and that the relative change in function after nephrectomy was also greater in weanling rats than in adults. C\(_{i/n}\)/KW in the experimental kidney was increased 4 wk after surgery, compared to 1 wk, in both age groups. These differences, however, were not significant.

Although previous reports have attempted to compare the compensatory response after uninephrectomy in children to adults, it is not possible to conclude that the adapted change in the young sexually immature child is similar to the experimental animal since careful pre-surgical assessment of renal function was not recorded and, in cases of Wilm’s tumor, the remaining kidney was treated with irradiation (16, 21). In a study of adult kidney donors for transplantation, examined on the average of 34 mo postnephrectomy, age at the time of surgery was correlated with the change in renal function pre and post surgery (17). Although a significant inverse correlation was found between age and increase in effective renal plasma flow, a similar relationship was not found for glomerular filtration rate. It should be possible to determine the age-related functional response to unilateral nephrectomy in man in future studies through careful assessment of pre-operative as well as post-operative values of renal function.

While these experiments demonstrate that the magnitude of the compensatory response is age dependent the mechanism for this change remains unclear. In previous studies from this laboratory it was shown that compensatory renal hypertrophy is not dependent upon the amount of work performed by renal tubules, reflected by the absolute sodium reabsorbed (24), but is stimulated in some way by the amount of tissue ablated or destroyed by disease (12). The present data suggests that the underlying type of normal growth is also an important determinant. As in normal growth, compensatory renal growth in the weanling period is characterized chiefly by hyperplasia and is greater than in the sexually mature rat which responds primarily by hypertrophy (4, 10). It is of interest that, once initiated, this greater response in weanling animals persisted into the hypertrophic phase of normal growth.

REFERENCES

1. Allen, R. B., and Mann, F. C., Experiments on compensatory renal hypertrophy. Arch. Pathol. 19, 341 (1935).
2. Altman, P. L., and Dittmer, D. S., “Growth, including Reproduction and Morphological Development,” Fed. Amer. Soc. Exp. Biol., Washington, DC, 1962.
3. Barrows, C. H., Jr., Roeder, L. M., and Olewine, D. A., Effect of age on renal compensatory hypertrophy following unilateral nephrectomy in the rat. J. Gerontol. 17, 148 (1962).
4. Brasel, J. A., Age dependent difference in DNA polymerase activity following uninephrectomy in rats. *Growth* **36**, 45 (1972).

5. Braun-Menéndez, E., and Chiodi, H., La funcion renal en la rata blanca despues de la nefrectomia unilateral. *Rev. Soc. Argent. Biol.* **23**, 21 (1947).

6. Dicker, S. E., and Shirley, D. G., Compensatory renal growth after unilateral nephrectomy in the new-born rat. *J. Physiol.* **228**, 193 (1973).

7. Farris, E. J., and Griffith, J. Q., “The Rat in Laboratory Investigation," Hafner, New York, 1962.

8. Halliburton, I. W., The effect of unilateral nephrectomy and of diet on the composition of the kidney. In “Compensatory Renal Hypertrophy" (W. W. Nowinski and R. J. Goss, Eds.), p. 101–130. Academic Press, New York, 1969.

9. Jackson, C. M., and Shielis, M., Compensatory hypertrophy of the kidney during various periods after unilateral nephrectomy in very young albino rats. *Anat. Rec.* **36**, 221 (1927).

10. Karp, R., Brasel, J. Q., and Winick, M., Compensatory kidney growth after uninephrectomy in adult and infant rats. *Amer. J. Dis. Child.* **121**, 186 (1971).

11. Katz, A. I., and Epstein, F. H., Relation of glomerular filtration rate and sodium reabsorption to kidney size in compensatory renal hypertrophy. *Yale J. Biol. Med.* **40**, 222 (1967).

12. Kaufman, J. M., DiMeola, H. J., Siegel, M. J., Lytton, B., Kashgarian, M., and Hayslett, J. P., Compensatory adaptation of structure and function following progressive renal ablation. *Kidney International* **6**, 10 (1974).

13. Krohn, A. G., Ogden, D. A., and Holmes, J. H., Renal function in twenty-nine healthy adults before and after nephrectomy. *J. Amer. Med. Ass* **196**, 322 (1966).

14. MacKay, E. M., and MacKay, L. L., Factors which determine renal weight. VII. Protein intake and age. *J. Nutrition* **3**, 375 (1931).

15. MacKay, E. M., MacKay, L. L., and Addis, T., The degree of compensatory renal hypertension following unilateral nephrectomy. I. The influence of age. *J. Exp. Med.* **56**, 255 (1932).

16. Mitus, A., Tefft, M., and Fellers, F. X., Long-term follow-up of renal functions of 108 children who underwent nephrectomy for malignant disease. *Pediat.* **44**, 912 (1969).

17. Ogden, D. A., Donor and recipient function 2 to 4 years after renal homotransplantation. *Ann. Int. Med.* **67**, 998 (1967).

18. Parks, J. R., Growth curves and the physiology of growth. II. Effects of dietary energy. *Amer. J. Physiol.* **219**, 837 (1970).

19. Parks, J. R., Growth curves and the physiology of growth. III. Effects of dietary protein. *Amer. J. Physiol.* **219**, 840 (1970).

20. Peters, G., Compensatory adaptation of renal functions in the unanesthetized rat. *Amer. J. Physiol.* **205**, 1042 (1963).

21. Raynaud, C., Ricard, S., Karam, Y., and Kellershohn, C., The use of the renal uptake of $^{32}$P as a method for testing the functional value of each kidney. *J. Nucl. Med.* **11**, 125 (1970).

22. Snedecor, G. W., and Cochran, W. G., “Statistical Methods,” 6th ed. Iowa State College Press, Ames, IA, 1967.

23. Winick, M., and Noble, A., Quantitative changes in DNA, RNA and protein during prenatal and postnatal growth in the rat. *Develop. Biol.* **12**, 451 (1965).

24. Weinman, E. J., Renquist, K., Stroup, R., Kashgarian, M., and Hayslett, J. P., Increased tubular reabsorption of sodium in compensatory renal growth. *Amer. J. Physiol.* **224**, 565 (1973).