Urological Research 3, 169-175 (1975)

Compensatory Renal Hypertrophy I. Following Unilateral Nephrectomy

An Experimental Study in Dogs

S. Olesen and P. O. Madsen

Received: June 11, 1975; and in revised form: September 29, 1975

Urology Section, Veterans Administration Hospital, and Department of Surgery,
University of Wisconsin School of Medicine, Madison, Wisconsin, U. S. A.

Summary. The compensatory renal hypertrophy and hyperfunction following unilateral nephrectomy have been studied in dogs over a two-year period. It was found that a sharp increase in function and volume of kidney tissue takes place during the first two weeks. C_{Cr} and volume of kidney tissue continue to increase reaching average values after two years of 88 % and 92 % respectively of those for two kidneys. C_{PAH} and Tm_{PAH} remain almost stable at 65 % and 57 % respectively after an initial increase during the first two weeks. All values are expressed in percent of the function of two normal kidneys before unilateral nephrectomy. The filtration seems to be increasingly efficient and the functional integrity of the nephrons is preserved, although a glomerular predominance is encountered two years following unilateral nephrectomy.

Key words: Compensatory renal hypertrophy, Dogs, Hydronephrosis, Renal clearances.

The urologist is often faced with the problem: "Is the kidney worth saving, and if not, is the other kidney able to take over the function?"

In unilateral kidney disease, the diseased kidney can be removed without doing any immediate harm to the patient. According to Hinman's counterbalance theory (9), the recovery of a hydronephrotic kidney following release of the obstruction will not be maximal if the opposite kidney is functioning normally. In bilateral kidney disease, the problems are more complex. The compensatory renal hypertrophy and hyperfunction is a great help to the urologist, but its importance is more easily appreciated if we speak and think in terms of nephrons rather than kidneys. Hayman et al. (1939), correlated renal function and nephron population (8). Platts (1952) elaborated the structural and functional adaptation in renal failure (16). In 1960 Bricker proposed the "intact nephron hypothesis" according to which no diseased nephron participates detectably in renal function (3). According to this hypothesis azotemia will occur only when a decreased population of normal nephrons is no longer able to handle the balance of electrolytes and water and the excretion of waste products.

Microdissection studies have documented that hypertrophy occurs in the remaining nephrons following reduction of the nephron number, regardless of whether the reduction was caused by surgical reduction (10), Bright's disease (15), or chronic hydronephrosis (2). It has also been documented, though not as widely accepted, that in Bright's disease the functional integrity of surviving nephrons is preserved, just as it is when the reduction of the nephron number is caused by unilateral or subtotal nephrectomy.

This study has been undertaken in order to determine the exact period of time required by the solitary kidney to reach its highest degree of compensatory hypertrophy and hyperfunction, and to investigate the functional patterns of the hypertrophied nephrons in solitary kidneys.

Material and Methods

Eight female mongrel dogs weighing from 10-20 kg ranging in age from 1-5 years were used in this study. The dogs were fed the same diet throughout the study. Four dogs were followed for two years and four for four months.

Unilateral nephrectomy was performed transperitoneally under pentobarbital anaesthesia. The length, width and depth of the removed kidneys were measured and the volume determined according to the method described in a previous publication (13). At the end of the study the hypertrophied kidneys were removed and measured in the same way.

Clearance Methods

Glomerular filtration rate was determined by the clearance of exogenous creatinine (C_{Cr}) or I^{125} labelled iothalamate. Effective renal plasma flow and the functioning tubular mass were determined by the clearance of p-aminohippuric acid (C_{PAH}) and the maximal excretory capacity for p-amino hippuric acid (Tm_{PAH}) respectively. The ability to handle the balance of water and electrolytes in the body fluid was measured by osmolar clearance (C_{OSM}), maximum concentration ability, sodium and urea excretion, and urine flow per minute. In addition, urinalysis for albumin, sugar, blood and pus cells, and fasting blood levels of creatinine, urea nitrogen, sodium and potassium were carried out.

Clearance Observations

Four-hour clearance observations were carried out before and at regular intervals after unilateral nephrectomy. The first clearance observation after unilateral nephrectomy was performed two weeks after the operation, allowing the animals complete recuperation. The animals were kept fasting for 20 hours before the clearance observations. Intravenous pentobarbital anaesthesia (25 mg/kg body weight) was used exclusively and maintained by infusion of 5-10 mg of pentobarbital every 45 min throughout the 4-hr observation period. A free airway was assured by tracheal intubation. Under these conditions, pentobarbital anaesthesia seems to be a safe anaesthetic for renal function studies in dogs (5).

Urine was collected through an indwelling 14F foley catheter. The urine produced immediately after complete emptying of the bladder following 20 hrs of dehydration was collected for determination of maximum concentrating ability and for urinalysis (protein, sugar and pus cells).

An indwelling intravenous polyethylene catheter was used for drawing of blood samples. Infusions were given in a different vein away from the one used for blood samples.

A moderate diuresis, usually 0.5-2.5 ml/min was induced by a priming dose of 300 mg mannitol/kg body weight and maintained by intravenous infusion of a 5 % mannitol solution at a rate of 2 ml/min (19).

The methods of Smith (1956) for determination of C_{Cr}, C_{PAH} and Tm_{PAH} in dogs were used: constant intravenous infusion of creatinine, and constant intravenous infusion of PAH were used to raise the blood concentrations to the recommended stable levels of 10-15 mg% creatinine and 1.5-2.5 mg% PAH (19). The isotope technique used to determine the GFR was that described by Oester, Olesen and Madsen (1968) (12). After a 45 min equilibration period, the urine was collected for consecutive periods of 15 min during each of which three blood specimens were taken. After the first three clearance periods, the blood concentration of PAH was raised by further intravenous infusion to approximately 25 mg% for determination of Tm_{PAH}. The entire procedure was then repeated. C_{OSM}, urine flow and excretion of sodium and urea determined in the first three clearance periods. The true mean concentration of a solute over any given interval of time was obtained by semilogarithmic interpolation.

The evacuation of the bladder was aided by placing manual pressure over the bladder area and by rinsing with 10 ml of distilled water followed by 10 ml of air.

Analytical Methods

Creatinine and PAH were determined by the methods of Folin and Wu (1919) (6) and Smith, Finkelstein, Alminosa, Crawford and Graber (1945) (18), respectively, using a Coleman spectrophotometer. Osmolality was determined by an Advanced Osmometer. An Auto-analyser was used for analyses of sodium, potassium and urea. A Packard well counter with window setting for I^{125} was used for determination of I^{125} iothalamate.

Intravenous pyelography has only been used to estimate renal size. Pyelograms were taken 3 and 5 min following intravenous injection of 1 ml of 50 % sodium diatrizoate/kg body weight and the renal size was estimated by cubing the maximal renal length as seen in the pyelograms. The volume of the kidney was found by means of a nomogram (13).

Results

Normal Values

All values for C_{Cr}, C_{PAH} and Tm_{PAH} are corrected to 1 sq. m surface area using a nomogram (17). The average values in 20

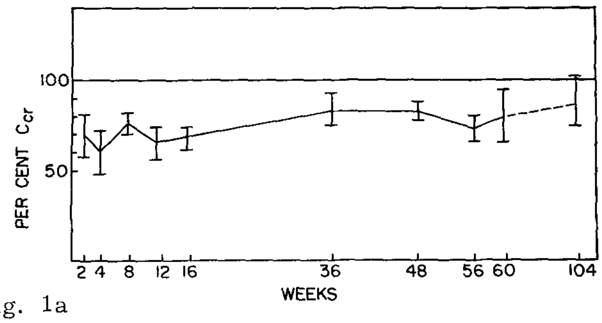

Fig. 1a

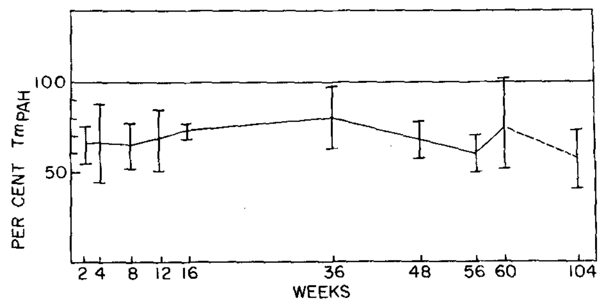

Fig. 1b

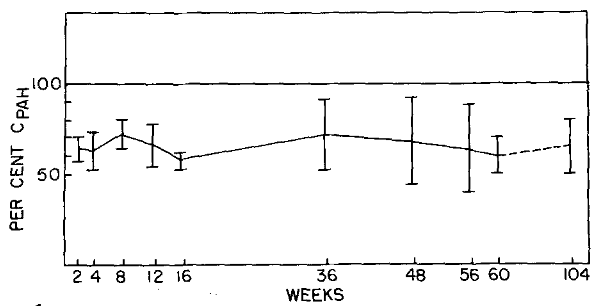

Fig. 1c

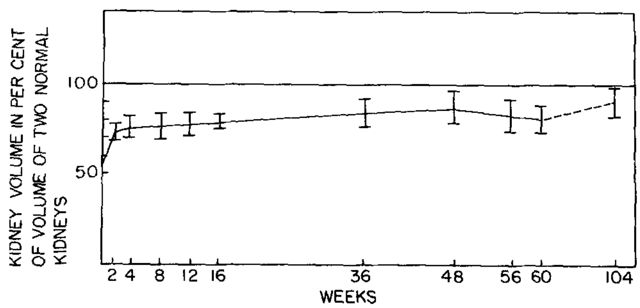

Fig. 1d

Fig. 1a, b, c, d. The percentage change in C_{Cr}, Tm_{PAH}, C_{PAH} and kidney volume illustrating compensatory renal hypertrophy following contralateral nephrectomy in eight dogs. The 100 % level is that of two kidneys (mean $\pm$ 1SD)

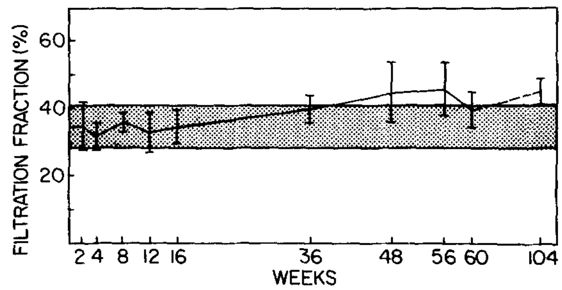

Fig. 2. The filtration fraction C_{Cr} X $100/C_{PAH}$. Hatched area indicates the normal range

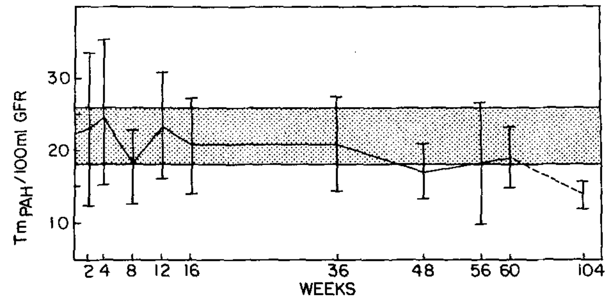

Fig. 3. Maximum tubular function per unit of C_{Cr}

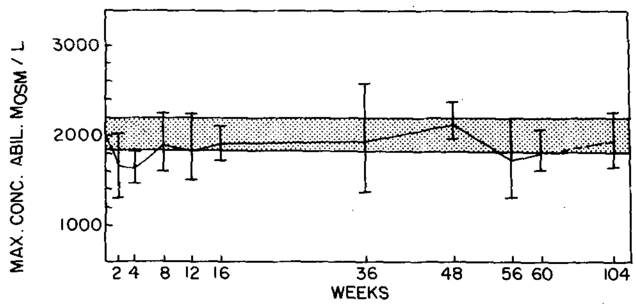

Fig. 4. The maximum concentrating ability

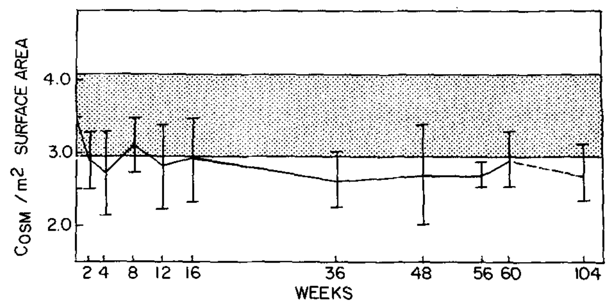

Fig. 5a. Osmolality clearance per sq m surface area

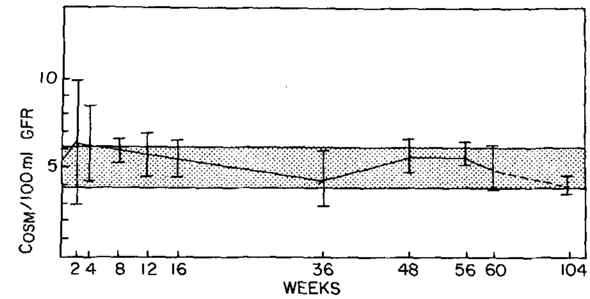

Fig. 5b. Osmolality clearance per unit of C_{Cr}

normal dogs with two kidneys were found in our laboratory to be:

C_{Cr}	- 74.2 ml/min $\pm$ 13.6	per sq meter surface area
C_{PAH}	- 218.3 ml/min $\pm$ 16.3	
Tm_{PAH}	- 16.4 mg/min $\pm$ 1.2	

This is in fairly good agreement with the results of other investigators (20, 1).

The percentage change in C_{Cr}, C_{PAH}, Tm_{PAH} and estimated renal volume is shown in Figs. 1a, b, c and d. There is a sharp increase in all four parameters from the theoretical 50% level during the first two weeks after which a slower increase seems to continue in C_{Cr} and kidney volume. Applying Student's t-test to the paired values of the four dogs followed for two years shows that the increase in both parameters from two weeks to two years is significant at the 95% level. That the kidney volume actually increased to an average value of 92% was confirmed by removal of the kidneys at the end of the study. C_{PAH} and Tm_{PAH} remain almost unchanged after two weeks with standard errors increasing during the study.

The filtration fraction (Fig. 2) was within normal limits throughout the first four months. A statistically significant increase took place during the rest of the observation period (Student's t-test for paired values: $p < 0.001$). The maximum tubular function per unit of C_{Cr} showed a downward trend throughout the study (Fig. 3). Three kidneys showed tubular predominance after two weeks, two after four weeks, one after twelve weeks, one after sixteen weeks and one after 36 weeks. During the second year, no tubular predominance developed, and at the end of the study, a significant glomerular predominance had taken place in all four kidneys (Student's t-test for paired values: $p < 0.001$).

The maximum concentrating ability was significantly lowered during the first month (Fig. 4). During the rest of the observation period, the kidneys were able to concentrate the urine normally (Student's t-test for paired values at two and four weeks; $p < 0.05$ and < 0.01 respectively).

In Fig. 5a, the C_{OSM}/sq m surface area is illustrated. This parameter was significantly lowered throughout the study. However, the C_{OSM} per unit of C_{Cr} is within normal limits (Fig. 5b).

A normal solitary kidney reabsorbs less sodium especially during the first two months following unilateral nephrectomy (p values for non-paired experiments at two, four and eight weeks < 0.02) (Fig. 6a). Due to high standard deviations, no statistical significance can be obtained from the changes of fractionated sodium excretion (Fig. 6b), but there seems to be an increased sodium excretion per nephron.

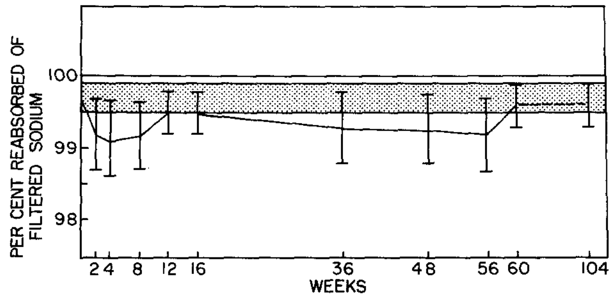

Fig. 6a. Percent of filtered sodium reabsorbed

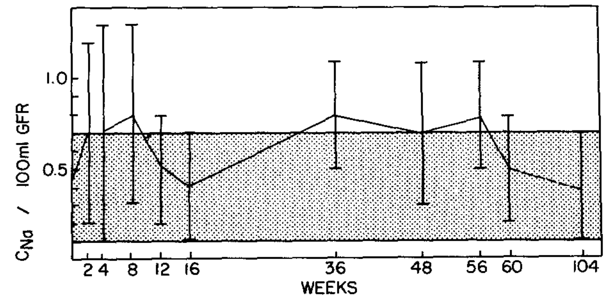

Fig. 6b. Fractionated sodium excretion

The fasting blood levels for creatinine, blood urea nitrogen, sodium and potassium remained within normal levels throughout the study.

The dogs stayed healthy during the entire observation period, and urinalysis did not reveal any abnormalities.

Discussion

A sharp increase in function and kidney size from a theoretical 50% level occurred during the first two weeks followed by a continued, slower, increase in C_{Cr} and kidney size. C_{PAH} and Tm_{PAH} remain almost unchanged after two weeks. Thus an increased filtration fraction and a significant glomerular predominance was found in four dogs after two years. There is a discrepancy between these results and those of Kolberg (1959) (10) and Maluf, Ford and Spurr (1957) (11) who found tubular predominance in experimental and human studies, respectively. It seems unlikely that an analytical error was responsible for the results of the present study. In an effort to find more reliable and less time-consuming clearance methods suitable for our laboratory, the clearances of exogenous creatine, inulin and I^{125} iothalamate were compared (Oester, Olesen and Madsen, 1968) (12). It was found that the clearance of creatinine when analysed according to the method of Folin-Wu, was consistently 40 % higher than the simultaneous clearances of inulin or I^{125} iothalamate. Owen, Iggo, Scandrett and Stewart (1954) postulate that this is caused by incomplete recovery of creatinine from plasma when pH of the filtrate is 3-4.5 (14). However, in the modified Owen-method, where the pH of the filtrate is below 2, 100 % of the creatinine will be recovered. This has been confirmed in our laboratory. Unfortunately, we were not aware of this when the present investigation was started. Our clearances of creatinine are therefore too high, but this is the case throughout the study, and the percentage change of the GFR and the changes of other parameters calculated by the GFR will therefore be reliable when compared to the normal averages found by the same method in our laboratory. The last clearance observation is made using the clearance agent I^{125} iothalamate instead of creatinine. Multiplying the clearances of I^{125} iothalamate with the factor 1.38, the C_{Cr} will be obtained, the error by doing so being only 5 %. The low value for $Tm_{PAH}/100ml$ GFR at two years (Fig. 3) could be due to artificially high values for creatinine clearances caused by calculation from the clearances of I^{125} iothalamate, but even a maximum error of 5 % in all C_{Cr} values would not produce significant alteration. It should also be mentioned that the average plasma concentrations of PAH during the Tm-periods of the clearance observation after two years were 19.4, 14.8, 25.3 and 17.8 mg% respectively in the four dogs. Thus, no artificial depression could have occurred. Apart from the infusion of iodine-125-iothalamate instead of 8 % creatinine solution, the clearance procedures were not changed.

Bugge-Apserheim and Kiil (1968) examined the renal function after contralateral uretero-peritoneostomy (functional nephrectomy) and after unilateral nephrectomy in dogs. The observation periods were about 4 weeks after each procedure (4). No uniform functional pattern as to glomerular or tubular predominance was found.

Accepting Kolberg's conclusion that there is a functional tubular predominance in hyperplastic nephrons as long as the hyperplasia is relatively undisturbed by other pathological processes, one may postulate that our dogs were suffering from chronic Bright's disease seen in dogs as they become older. As mentioned above, the urinalysis did not reveal any abnormality. Although the exact age of the dogs was unknown, none were more than eight years old, and microscopic examination of the kidneys at the end of the study showed no pathological changes, such as chronic infection. We must therefore conclude that the disproportion between C_{Cr} and Tm_{PAH} with glomerular predominance encountered in this study after two years is not caused by observational or analytical errors, or by renal disease.

Figure 7 shows the relationship between the so-called ischaemia factor C_{PAH}/Tm_{PAH} and the filtration fraction C_{Cr}/C_{PAH} (20). The data by which the curve is constructed are obtained from observations in seventeen normal dogs. The solid curve represents the course of the filtration fraction when the filtration rate is constant, i. e., if

$$\frac{C_{Cr}}{C_{PAH}} \times \frac{C_{PAH}}{Tm_{PAH}} = \frac{C_{Cr}}{Tm_{PAH}} = 4.6$$

The dotted lines represent this mean value $\pm$ 0.8. The hexagon is an arbitrary area containing all normal data obtained under "basal" conditions. This ratio reflects changes in both the nephrons and the vessels. 80 % of the values obtained during the first sixteen weeks after unilateral nephrectomy fall within the hexagon. Between nine months and two years, however, 80 % of the values are scattered to the right of and above the hexagon, which indicates that there is a statistically significant increase in both the filtration fraction and the ischaemia factor, the p values being less than 0.001 and 0.05 respectively.

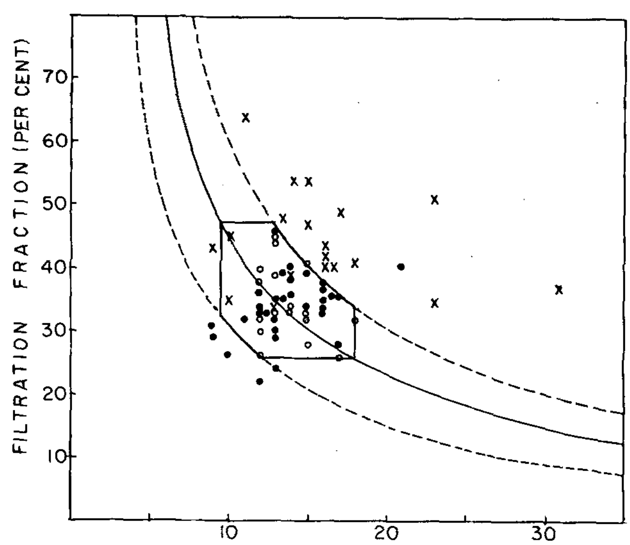

Fig. 7. The filtration fraction in relation to renal plasma flow per unit of Tm_{PAH}, O : normal values, ● : values obtained during 2-16 weeks after unilateral nephrectomy X : values obtained 36-104 weeks after unilateral nephrectomy.

According to Bugge-Asperheim and Kiil (1968) the compensatory growth is dependent upon loss of renal mass per se and not related to an increased workload (4). The maximum transportation of glucose and p-amino hippuric acid is growth dependent, whereas the changes in C_{Cr}, C_{PAH} and tubular sodium reabsorption and the regulation of sodium excretion seem to be adaptive.

It is well-known that the compensatory hypertrophy is more pronounced in the proximal tubules than in other parts of the nephrons (15, 10). We determined C_{Cr}, C_{PAH} and Tm_{PAH} per cm^3 of renal tissue in four dogs observed for 2 years after unilateral nephrectomy. C_{Cr} rose from 0.76 to 2.24 $ml/min/cm^3$ and C_{PAH} from 2.41 to 2.56 $ml/min/cm^3$. The increases are statistically significant, p values being less than 0.05 by a t-test for paired values. On the other hand Tm_{PAH} per unit of renal tissue did not change at all, the values before and after unilateral nephrectomy being 0.15 ± 0.04 and 0.16 ± 0.06 $mg/min/cm^3$ respectively. These findings might well support the findings of Bugge-Apserheim and Kiil (4) that the maximum transport of p-amino hippuric acid is solely growth dependent - and other factors are responsible for the change in C_{Cr} and C_{PAH}. Thus the functional pattern of tubular or glomerular predominance after loss of nephrons would not be determined by the loss of nephrons per se, but more likely by the length of observation, experimental model etc.

The ability of hydropenic dogs with a 50% nephron loss to concentrate the urine maximally is intact one month following unilateral nephrectomy, and the individual nephrons are able to excrete osmotically active substances in a normal way. During osmotic diuresis the osmolar clearance of one kidney is significantly lowered, but per unit of C_{Cr} it is within the normal range throughout the 2-year observation period. This indicates that the clearance of osmotically active substances is quite normal in the residual nephrons no matter whether the observation is made 2 weeks or 2 years after a 50% nephron loss.

From our data, it is not possible to explain the increased sodium excretion during the first 2 months. Because of the relatively small pathophysiological variations in the clearance of sodium as compared to the analytical errors in the clearance of creatinine, the fractionated sodium excretion will give us no information as to the sodium excretion of the individual nephron.

Our experimental model was not designed for an investigation of sodium and water transport. The present study shows that the essential morphological and functional changes in a kidney after contralateral nephrectomy take place during the first two to three months whether the changes are adaptive or growth dependent. The functional integrity of the nephrons is preserved in either case. If this intact nephron hypothesis is applicable to any chronic renal disease we should see many patients with identical functional patterns since compensatory hypertrophy would take place in residual nephrons no matter how the nephron loss came about.

References

1. Åsheim, Å., Persson, F., Persson, S.: Renal clearance in dogs with regard to variations according to age and sex. Acta Physiol. Scand. 51, 150 (1961)
2. Bialestock, D.: Microdissection of specimens of hydronephrosis with comparative nephron measurements. Aust. N.Z. J. Surg. 29, 211 (1960)
3. Bricker, N.S., Morin, P.A.F., Kime, S. W., Jr.: The pathologic physiology of chronic Bright's disease: An exposition of the "Intact Nephron Hypothesis". Amer. J. Med. 28, 77 (1960)
4. Bugge-Asperheim, B., Kiil, F.: Examination of growth-mediated changes in haemodynamics and tubular transport of sodium, glucose and hippurate after nephrectomy. Scand. J. Clin. Lab. Invest. 22, 255 (1968)
5. Corcoran, A.C., Page, I.H.: Effects of anesthetic dosage of pentobarbital sodium on renal function and blood pressure in dogs. Amer. J. Physiol. 140, 234 (1943)
6. Folin, O., Wu, H.: A system of blood analysis. J. Biol. Chem. 38, 81 (1919)
7. Glauser, K.F., Selkurt, E.E.: Effect of barbiturates on renal function in the dog. Amer. J. Physiol. 168, 469 (1952)
8. Hayman, J.M., Jr., Shumway, N.P., Dumke, P., Miller, M.: Experimental hyposthenuria. J. Clin. Invest. 18, 195 (1939)
9. Hinman, F.: Renal counterbalance. J. Urol. 9, 289 (1923)
10. Kolberg, A.: Relations of renal tubular and glomerular function as influenced by 75% reduction of nephron number. A pathophysiological study. Scand. J. Clin. Lab. Invest. (Suppl. 41). 11, 1 (1959)
11. Maluf, N.S.R., Ford, R.V., Spurr, C.S.: Physiology of the human solitary kidney. J. Urol. 78, 118 (1957)
12. Oester, A., Olesen, S., Madsen, P.O.: Determination of glomerular filtration rate. Old and new methods. A comparative study in dogs. Invest. Urol. 6, 315 (1968)
13. Olesen, S., Genster, H.: Estimation of renal volume and function in dogs from the radiological appearance. Invest. Urol. 7, 363 (1970)

14. Owen, J.A., Iggo, B., Scandrett, F.J., Stewart, C.P.: The determination of creatinine in plasma or serum, and in urine; a critical examination. Biochem. J. 58, 426 (1954)
15. Oliver, J.: When is a kidney not a kidney? J. Urol. 63, 373 (1950)
16. Platt, R.: Structural and functional adaption in renal failure. Brit. Med. J. 1952 I, 1313
17. Sendroy, J., Jr., Cecchini, L.P.: Determination of human body surface area from height and weitht. J. Appl. Physiol. 7, 1 (1954)
18. Smith, H.W., Finkelstein, N., Alminosa, L., Crawford, B., Graber, M.: The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. Clin. Invest. 24, 388 (1945)
19. Smith, H.W.: Principles of renal physiology. New York: Oxford University Press 1956
20. Smith, H.W.: The kidney. Structure and function in health and disease. 3rd Ed. 1958 New York: Oxford University Press

Sigurd Olesen, MD,
Bjoernkaervej 2
DK-8471 Sabro
Denmark