The Influence of Age on the Response to Renal Parenchymal Loss

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The effect of age on compensatory hypertrophy and functional adaptation to loss of 75 percent of renal mass was studied in canine puppies. In one group of animals the surgery was done between 1-5 days after birth and in another group, at two months of age. All animals were studied six weeks later. Sham-operated littermates served as controls. The newborn puppies in the experimental group were able to grow and maintain homeostasis as well as their controls, whereas the older experimental animals grew poorly and had significantly higher levels of plasma creatinine than their sham-operated counterparts \((p < .05)\). The increase in mass of the remaining kidney was twice as much in the newborn as in the older dogs. Functional adaptation, as expressed by GFR, was nearly complete in the young, but reached only about 45 percent of controls in the older age group \((p < .005)\). The intrarenal blood flow distribution was similar for experimental and control animals in both groups studied. There were, however, marked differences in the pattern of single glomerular perfusion rates: whereas in the older dogs the increase was confined to the deeper nephrons, in the newborn an increase occurred in all zones of the kidney. These studies demonstrate that compensation for massive loss of renal tissue is complete when the injury is sustained in the immediate postnatal period but only partial when it occurs later on in life. A loss in the adaptive capacity of the superficial nephrons appears to account for this age-related difference.

INTRODUCTION

The degree of compensatory hypertrophy in response to decrease in renal mass is related to the intrinsic capacity of the kidney for growth. If uninephrectomy is performed in rats soon after birth, the ensuing compensatory renal growth is the result mainly of an increase in cell number (hyperplasia). After this period, an increase in cell size (hypertrophy) proceeds concomitantly and becomes predominant by 40 days of age [1]. The ability to draw upon both these processes probably accounts for the enhanced capacity of the immature animal to compensate morphologically more fully than the adult [2-4]. There is some clinical evidence that the functional compensation is also more complete when the loss of kidney parenchyma occurs very early during development. Indeed, it appears that the only subjects with one kidney reaching values of GFR comparable to those of normal individuals are those with congenital unilateral renal agenesis [5]. On the other hand, in a study done in rats, Galla et al. [6] were unable to document a significant difference in glomerular filtration rate, expressed per unit of kidney weight, between animals uninephrectomized during the weaning period and those subjected to similar surgery in their adult life.
Previous work performed in our laboratory has demonstrated that during early extraterrestrial life various populations of nephrons are at different stages of development, the superficial nephrons lagging behind the deeper nephrons [7-9]. It is conceivable that the resulting heterogeneity might affect the contribution of various populations of nephrons to the compensatory process.

We addressed these problems by studying the changes in overall renal function, the intrarenal distribution of blood flow and the distribution of glomeruli in puppies following a 75 percent reduction in renal mass immediately following birth and comparing the results to those observed in a group of dogs who underwent the same procedure at two months of age.

METHODS

The animals were anesthetized with ether and the left kidney was exposed through an abdominal incision. The vascular pedicle was dissected free of fat and the renal artery with its main branches were exposed at the hilus. Primary branches providing blood supply to approximately 50 percent of the left kidney were ligated. After closure of the abdominal wound, the right kidney was approached through a flank incision, the vascular pedicle and ureter ligated and the kidney removed. Sham-operated littermates served as controls. Following the procedure, the newborns were returned to their mothers, whereas the older animals were returned to their cages and were fed commercial food supplemented by milk. Measurements of body weight, hematocrit, serum Na, K, Cl, CO₂, BUN and creatinine were performed weekly.

Six weeks later the animals were anesthetized with thiopental sodium, the ureter, right femoral artery and vein were cannulated, the left ventricle was catheterized via the left carotid artery and measurements of GFR (by standard inulin clearance), RPF (by clearance of PAH corrected for extraction), and intrarenal distribution of blood flow (by the microsphere method) were obtained. For this latter procedure the cortex was divided in four equally wide zones. The subcapsular area was designated zone I and the juxtamedullary cortex zone IV.

Glomerular counts were performed on photomicrographs of transverse sections on which the cortex was divided in four strips, corresponding to those used for measurements of intrarenal distribution of blood flow.

The weight of each zone was calculated according to the method of McNay and Abe [10]. Finally, using the glomerular number per zone and the absolute blood flow per zone, the rate of perfusion per glomerulus in each zone could be estimated.

RESULTS

Table 1 shows the results of baseline measurements at six weeks of age in experimental puppies and their control littermates. The experimental animals were able to grow well and to maintain homeostasis, despite a massive destruction of renal parenchyma. The only significant difference between the two groups was in kidney mass: the remaining, partially infarcted kidney in the experimental animals reached 60 percent of the weight of the two normal kidneys of the controls.

When GFR and RBF were calculated per gram of kidney, no significant differences were observed, although a trend towards higher values was evident in the experimental animals, suggesting that the functional adaptation exceeded that expected solely on the basis of compensatory hypertrophy. It should be pointed out that expressing this measurement per unit of weight obscures the increase in RPF and GFR per nephron. This is a consequence of the massive tubular hypertrophy which dilutes the density of the glomeruli. Assuming that no new nephrons were formed, the
GFR per nephron had to increase in the remnant kidney by more than 2.5-fold in order to account for the observed rise in total kidney GFR.

On the basis of body surface area, puppies which lost 75 percent of their kidney tissue were able to reach levels of GFR which were, on the average, only 15 percent lower than those of their intact littermates.

Unlike the newborns, significant differences between the control and experimental animals were consistently observed in the older dogs (Table 2). Animals in the experimental group had a significantly lower body weight than their control littermates and their serum creatinine was significantly elevated from a control value of $0.38 \pm 0.05$ to $0.51 \pm 0.01$ mg%.

The weight of the kidney was $47.9 \pm 5.8$ g in control animals with two kidneys and $24.2 \pm 1.2$ g in animals with a remnant kidney.

The level of GFR reached by the experimental animals was 45 percent that of the controls. Similarly, the rate of RPF in the remnant kidney was only 41 percent that observed in the control animals. When the results were expressed per gram of wet kidney weight, the differences in GFR and RPF between the two groups of animals became insignificant, the values tending to be higher in the control group.

There were only minor differences in the pattern of distribution of renal blood flow between various zones in either group. However, there were striking differences in the distribution of glomeruli between the various zones, in both groups, and these changes appeared to be greater and wider spread in the kidneys of the animals who suffered the injury at birth. Consequently, in this latter group the perfusion rates of single glomeruli determined from distribution of microspheres per gram of zone and the number of glomeruli per gram of zone, increased by about 300 percent in all but

| Table 2 Measurements at 14 Weeks of Age Following Surgery at 8 Weeks |
|---------------------------------------------------------------|
| **Control** | **Experimental** | **P** |
| (x ± SEM, n = 4) | (x ± SEM, n = 3) |     |
| Body weight (g) | 6,325 ± 626 | 4,700 ± 252 | <.05 |
| Kidney weight (g) | 47.9 ± 5.8 | 24.2 ± 1.2 | <.005 |
| P_{cr} (mg/100 ml) | 0.38 ± 0.05 | 0.51 ± 0.01 | <.05 |
| GFR (ml/g kidney) | 1.2 ± 0.2 | 0.9 ± 0.1 | <.2 |
| GFR (ml/min/g m²) | 161.1 ± 12.3 | 73.2 ± 9.0 | <.005 |
| RPF (ml/min/g kidney) | 4.3 ± 0.7 | 2.8 ± 0.3 | <.2 |
| RPF (ml/min/m²) | 586.4 ± 81.3 | 239.3 ± 27.2 | <.005 |
were intrarenal arterioles and a 5-fold increase in kidney weight in the weight of the remaining tissue. By comparison, animals operated at 8 weeks experienced a 3.5-fold increase in the weight of their remaining kidney, whereas the sham-operated littermates doubled their renal mass during the same period of observation. Thus, compensatory growth accounted for a fivefold increase in the newborns and for only a 1.5-fold increase in kidney weight in the adult animals. This confirms in yet another animal species that the degree of compensatory renal hypertrophy is related to the intrinsic capacity of the kidney for growth which, in turn, is a function of age [1,2,3,6,11].

Original to the present study is the observation that functional adaptation is also more complete when the injury to the kidney occurs early in life. Whereas the animals which underwent a 3/4 reduction in renal mass at birth reached by six weeks of age a level of GFR not statistically different from their sham-operated littermates, the dogs operated at eight weeks had six weeks later a GFR which amounted to 45 percent of that observed in the controls. This remarkable degree of functional adaptation explains the fact that despite massive ablation of renal tissue, the newborn animals were able to maintain body fluid homeostasis and grow in weight to the same extent as their littermates. On the other hand, the older animals in the experimental group were smaller, and had higher serum creatinine than their counterparts.

The intrarenal blood flow distribution was similar for experimental and control animals in both age groups studied. Since, as we have shown previously [8,9], the intrarenal blood flow distribution in puppies reaches an adult pattern by six weeks of

| Zone | Surgery at Birth | | Surgery at 8 Weeks | |
|------|-----------------|-----------------|-----------------|-----------------|
|      | Control (n = 3) | Exper. (n = 5) | p               | Control (n = 4) | Exper. (n = 3) | p               |
| I    | 34.6 ± 2.0      | 109.8 ± 25.7    | >.05            | 408.3 ± 69.2    | 417.0 ± 21.4    | <.5             |
| II   | 27.8 ± 5.1      | 77.0 ± 12.1     | <.02            | 225.5 ± 39.1    | 275.3 ± 55.7    | <.5             |
| III  | 38.6 ± 4.4      | 90.6 ± 12.3     | <.02            | 151.3 ± 25.0    | 299.3 ± 33.3    | <.05            |
| IV   | 171.3 ± 79.1    | 115.1 ± 21.2    | <.4             | 148.3 ± 35.5    | 365.3 ± 84.2    | <.05            |

The relationship between the degree of renal compensatory hypertrophy and age was hypothesized almost 50 years ago [2]. However, this work and most of the succeeding studies [1,3,4,5,11] failed to distinguish between normal and adaptive growth and only one [6] of them explored the functional correlates of the age-dependent changes in morphology. The experiments presented in this report were designed to fill this gap.

Studies were performed during two distinct periods of postnatal renal development: the first week of life in which renal growth is characterized by a very active multiplication of cells and rapid functional changes, and the eighth week in which growth of the functionally mature kidney is slow [12]. Concomitantly, by using littermates as controls, we were able to compare compensatory hypertrophy to normal growth.

Control animals examined six weeks after birth experienced a 2.5-fold increase in kidney weight as a result of their normal growth whereas experimental animals showed a 7.5-fold increase in the weight of the remaining tissue. By comparison, animals operated at 8 weeks experienced a 3.5-fold increase in the weight of their remaining kidney, whereas the sham-operated littermates doubled their renal mass during the same period of observation. Thus, compensatory growth accounted for a fivefold increase in the newborns and for only a 1.5-fold increase in kidney weight in the adult animals. This confirms in yet another animal species that the degree of compensatory renal hypertrophy is related to the intrinsic capacity of the kidney for growth which, in turn, is a function of age [1,2,3,6,11].

DISCUSSION

The deepest nephrons. In the animals operated at two months the increases were significantly less and were confined only to the inner cortical nephrons (Table 3).
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It is not surprising that differences between control animals in the two age groups were not seen.

It is of interest that marked differences in the pattern of single glomerular perfusion were observed between the two groups, following reduction. In the older animals the increase was confined to the deeper nephrons. In contrast, in the newborn an increase in single nephron blood flow occurred in all zones, but particularly in the superficial area. This demonstrates that the less mature structures of the superficial zone [7,8,9] have an adaptive capacity which exceeds that of the deeper cortex, and that this capacity diminishes with aging.

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