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Induction of Extra Nephrons in Unilaterally Nephrectomized Immature Rats[1] (38525)

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Contrary to prevailing opinion on the matter, it has recently been discovered (1, 2) that the number of glomeruli that develop in the rat kidney can be increased above normal levels by performing unilateral nephrectomies in young animals. It is well established that in the adult rat, compensatory renal hypertrophy is not accompanied by increases in the number of nephrons (3, 4). Even in young rats, earlier investigators (5–7) had concluded that the remaining kidney could develop no more nephrons than they normally do. Hence, recent reports to the contrary deserve to be confirmed and amplified to prepare the way for future investigations of the physiological factors controlling nephrogenesis.

Materials and Methods. Sprague Dawley rats (Charles River CD) of both sexes were used in these experiments. Kidneys were removed through dorsolateral incisions from newborn rats immobilized by a 30 min exposure to 5°, or from older ones anesthetized with ether.

In a total of 14 litters, averaging 10 per litter, some animals were sacrificed at 10-day intervals from birth to 50 days, as well as at 70 and 100 days, as a source of kidneys from which the normal growth and nephron number could be determined during maturation. The remaining littermates were unilaterally nephrectomized on the same schedule up to 70 days of age and their removed kidneys were combined with those of the unoperated controls. The experimental rats were all sacrificed at 70 days of age, except for the ones nephrectomized at 70 days, which were sacrificed when they were 100 days old.

Removed kidneys were decapsulated and weighed prior to being prepared for glomerular counts according to a modification of established procedures (2, 8). Such kidneys were macerated in 50% HCl at room temperature for 24 hr and stored for up to 2 days in distilled water at room temperature until counted. Each kidney was made into a suspension in 50 ml of water by gently drawing it in and out of the wide end of a pipette until its tissue fragments were completely separated. A 0.1 ml sample of this suspension was drawn into a syringe and placed in a counting chamber. The sample was suspended freely between a microscope slide and cover glass at a depth of 1 mm without making contact with the edges of the chamber in order to avoid undue distortion. Glomeruli were then counted at 40X magnification, being made visible by the orange color of the residual blood they contained. Two samples of each kidney were averaged, and if their counts varied by more than 5%, a third sample was taken and all of them were averaged for a final estimate. The glomerular counts from such samples were then extrapolated to give a total glomerular count per kidney.

Results: A comparison of Figs. 1 and 2 reveal that although the absolute weight of the kidney continues to increase during the first 100 days of life, its relative weight begins to decline after the 10th day. Thus, the most rapid growth of the kidney occurs during the first 10 days after birth at which time they are increasing in mass at a rate greater than that of the body as a whole.

It is during this period that the number of glomeruli increases most rapidly. In agreement with earlier investigators (9, 10) we find that the newborn kidney contains approximately 10^4 nephrons. This number is more than doubled by 10 days of age and the adult complement of $34–35 \times 10^3$ nephrons is reached by 40 days (Fig. 3).

When one kidney is removed from the newborn rat, the remaining kidney at 70

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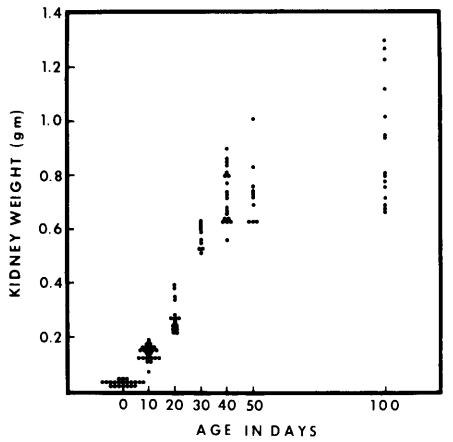

FIG. 1. Increase in absolute weight of rat kidneys as a function of age.

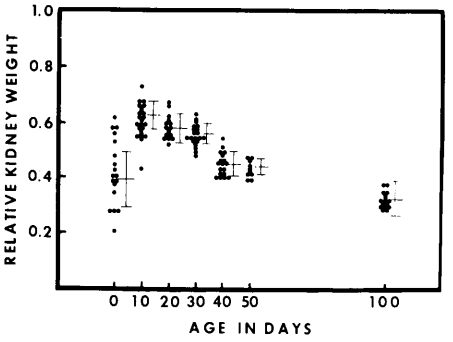

FIG. 2. Relative kidney weights [(kidney wt/body wt) × 100] in rats at different ages, including means ± SE.

days of age is found to contain approximately 57 × 10^3 glomeruli, representing a 63% increase over control values. Figure 4 illustrates how this increase in the complement of glomeruli is influenced by unilateral nephrectomies performed in progressively older rats. Up to the age of approximately 50 days their capacity to augment the adult population of nephrons is gradually abridged. Unilaterally nephrectomized 70-day old rats react as adults in that compensatory renal hypertrophy is accompanied by no further increase in nephron number.

Discussion. Normal kidney growth in the rat is achieved predominantly by cellular proliferation during the early stages of development and by cell hypertrophy at progressively later stages (11). At the tissue level of organization, the infant kidney continues to make new nephrons up to approximately 40 days of age, a process which cannot go on without the addition of new cells. Occurring principally in the outer

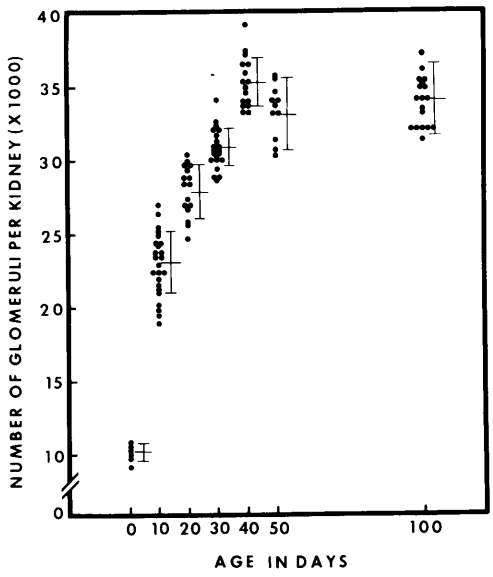

FIG. 3. Normal increase with age in the number of nephrons per kidney based on glomerular counts.

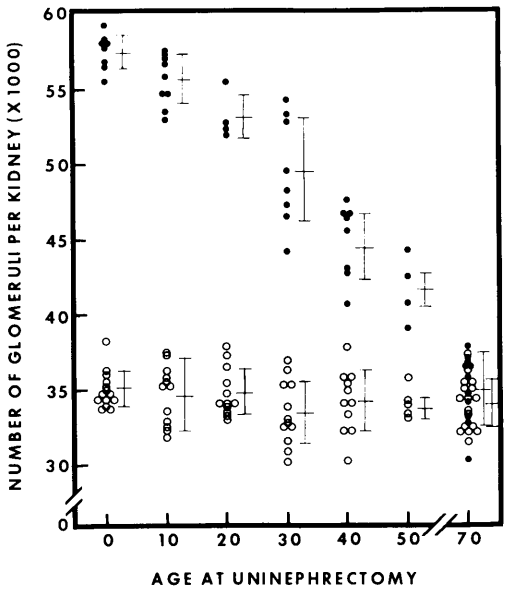

FIG. 4. Distributions of glomerular counts (solid dots) in rats unilaterally nephrectomized at different ages and sacrificed at 70 days. (Those operated at 70 days of age were sacrificed at 100 days.) Open circles indicate control values for unoperated littermates at 70 or 100 days, respectively.

nephrogenetic zone (12) the differentiation of nephrons in the developing kidney gradually decreases until the adult complement is attained. It is interesting to note, however, that even after this point there is approximately a 10 day period when the kidney is capable of increasing its population of nephrons above normal even though it does not ordinarily do so. Hence, unilateral nephrectomy of a 50-day old rat can still elicit a 20% increase in the number of glomeruli by 70 days of age. Thus, the potential for nephrogenesis seems to persist for a short time after the last nephron is produced.

In view of the present confirmation of the discovery by Bonvalet *et al.* (1) and Imbert *et al.* (2) that immature rat kidneys are in fact capable of making extra glomeruli, it is difficult to explain how others (5–7) failed to detect such a response in rats unilaterally nephrectomized at various ages from birth to 50 days. On the other hand, one would expect to find supernumerary nephrons in cases of the congenital absence of one kidney. According to Moore (13), this was indeed the case in a human subject, but Boycott (14) found no such result in a rabbit born with only one kidney, despite the fact that the remaining one had undergone considerable hypertrophy by the time it was examined in the adult.

The fact that compensatory growth of the kidney in immature rats is accompanied by the production of extra numbers of nephrons suggests that the species-specific complement of nephrons is not genetically determined. This possibility demands the search for physiological factors in the developing organism to which the production of nephrons adapts. In fishes, normally capable of adding new nephrons throughout life (15), there is evidence that the salinity of the water in which they are raised is inversely proportional to the number of glomeruli that develop in their kidneys (16, 17). Growth retardation in mice by hypoxia (18) or in rats by maternal malnutrition (19) also reduces the number of glomeruli per kidney. Clearly, the time is at hand to contemplate experiments designed to test a variety of physiological parameters for their ability to influence the number of nephrons that develop in the growing animal.

Summary. The normal number of glomeruli per kidney in the rat rises from about 10^4 at birth to approximately 35×10^3 at 50 days of age. When one kidney is removed at birth the remaining one produces an average of 63% more nephrons than normal by 70 days. Unilateral nephrectomy of successively older rats results in progressively less augmentation of the nephron complement in the remaining kidney up to 50 days, beyond which age the kidney loses its ability to produce new nephrons.

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