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Suppression of Cellular Proliferation in Weanling Rat Kidneys by Short-Term Fasting* (33068)

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Investigations performed in weanling and adult male rats disclosed that labeling with tritiated thymidine (TdR-^{3}H) and mitotic activity are observable in the normal kidney at all ages. The response to unilateral nephrectomy in weanling male rats is manifested by a transient sixfold increase in the labeling index (1). Other investigations (2) demonstrated that mitotic counts in the kidneys of female weanling rats were elevated markedly at 48 and 72 hours after unilateral nephrectomy. When food was withheld for 1, 2, or 4 days, mitotic activity was dramatically reduced in these animals. Similar depression following starvation was noted in mice by Reiter (3) and in rats by Williams (4). This reduction in proliferative activity occurred in the remaining kidneys of nephrectomized rats as well as in the kidneys of starved control rats. These results and those previously reported in association with radiation effects (5–7) suggested that decreased food intake may be a contributing factor in the decreased cell turnover in the kidney after total body irradiation.

The present investigation was designed to study the effects of starvation at frequent time intervals following its initiation in both intact and unilaterally nephrectomized animals. It was hoped that such a study would shed light on the possible control mechanism regulating renal cell proliferation.

Materials and Methods. Animals. Female Sprague-Dawley rats, approximately 30 days of age, weighing an average of 100 gm were used. All animals were kept in cages on wire mesh to prevent access to stool or urine when starved; however, access to water *ad libitum* was assured.

Surgical procedures. Nephrectomies were performed through an incision in the left flank. The kidney was stripped of its capsule and all extraneous tissue, weighed, and prepared for autoradiography. Autoradiographic studies were also made with bone marrow obtained from the femur in order to determine whether the starvation effects were nonspecific.

Autoradiography. Labeling of cells engaged in DNA synthesis was accomplished by intravenous injection of 0.5 μC/gm body weight TdR-^{3}H (specific activity = 1.9 C/mmole). Animals were killed 45 min after TdR-^{3}H injection. Kidney material was prepared for autoradiography as previously

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described (1). Bone marrow smears were coated with emulsion and prepared as the kidney sections with the exception that Wright's stain was used instead of hematoxylin and eosin.

The labeling index (percentage of cells labeled) was determined by counting cells in 50 random oil immersion fields (784 ×). All fields counted were in the cortical region of the kidney in zones adjacent to glomeruli. Standard deviations were calculated for the counts from control animals not subjected to nephrectomy. Because the experimental groups contained only 2–5, usually 3, rats, the complete range of cell counts is shown transformed into percentage. Bone marrow labeling was determined by counting the labeled cells in 1000 nucleated bone marrow cells.

Kidney DNA content. Kidneys of some control and food restricted rats were analyzed for total DNA content following homogenization employing the indole method of Ceriotti (8) as modified by Keck (9).

Experimental groups, Group I—controls: Labeling indices obtained from the kidneys of normally fed rats represent the base line for the experimental studies. Groups of 3–5 additional control animals were subjected to left nephrectomy and the remaining kidney removed and studied at intervals up to 1 week. Group II—starved only: Groups of 3 animals, allowed only water, were killed and their kidneys prepared at 4-hour intervals during the first 24 hours after starvation and at 48 hours. Group III—starved and refed: Animals were starved 20 hours then allowed food *ad libitum*. Groups of 3 animals were killed at 4-hour intervals for 24 hours after feeding resumed. Group IV—starvation plus nephrectomy: Animals were starved for 24 hours, a left nephrectomy was performed, and the animals were maintained without food for 72 hours. Groups of 3 were sacrificed at 8-hour intervals. Group V—low food: These animals were subjected to 24-hour starvation, nephrectomy, and continued starvation for a total of 96 hours. Small amounts of food (6 gm/animal per 24 hours) were then given for a total of 48 hours. This intake is sufficient to maintain but not to increase body weight during this time period.

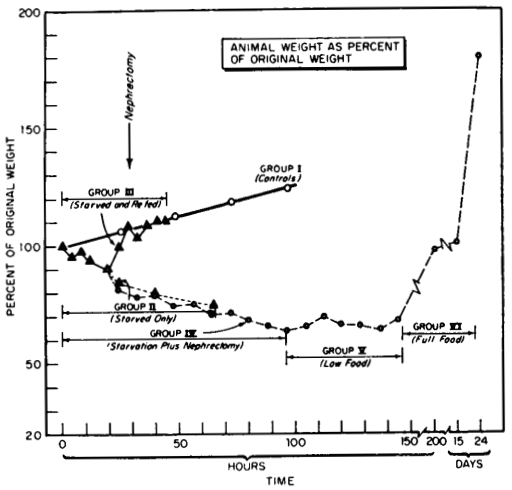

FIG. 1. Fluctuation in the weight of 100 gm, 30-day-old female rats after the various feeding procedures.

Animals were killed at 8-hour intervals. Group VI—full food: Rats were starved 24 hours, nephrectomized, starved 72 hours, and given low food for 48 hours. After resumption of feeding *ad libitum,* the animals were killed at 48 and 72 hours, 9 and 18 days. Group VII—low food only: Animals were studied for total DNA content of both kidneys. The rats were obtained at age 26 days and given 7 gm of food/day for 3 weeks (final age, 47 days). The DNA content of their kidneys was compared to that of rats fed a normal diet and sacrificed at the ages of 27, 37, and 47 days. Their kidney DNA content was also compared to that of a single kidney in normally fed animals of a similar age 3 weeks after a unilateral nephrectomy.

Results. Changes in animal weight. The animals not subjected to nephrectomy or starvation (Group I) gained weight at about 6 gm/day (Fig. 1). Withholding of all food caused a 35% weight loss in 4 days (Groups II and IV). The weight level remained unchanged for the next 2 days while the rats were limited to a restricted intake of food (Group V). Weight increased rapidly after food was replaced *ad libitum* (Group VI). Animals starved 20 hours then fed (Group III) rapidly returned to the control growth curve (Group I) when food was returned to the cages.

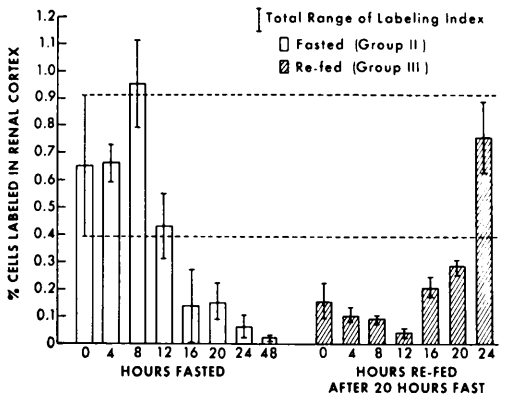

FIG. 2. Response of labeling index in kidney cells in nonnephrectomized rats to variation in food intake.

Changes in renal weight. Previous results indicated that renal weight is a constant fraction of body weight (5). Since a loss in body weight of starved rats resulted in a proportional loss in kidney weight, the ratio kidney weight to body weight remained constant within a range of ± 10% of the initial value in this study. In starved nephrectomized rats, a constant weight was maintained in the remaining kidney despite a drop in body weight resulting in an increase in the ratio of kidney to body weight.

Effects on labeling index. There was no marked alteration in the percentage of labeled kidney nuclei in intact animals 4–12 hours after fasting (Fig. 2). After 12 hours the labeling index decreased progressively. When feeding was resumed after 20 hours of fasting, the labeling index did not rise for 12 hours then reached the normal range after 24 hours. Tubular cells resumed DNA synthesis, and thus proliferation, earlier than stromal cells.

Proliferative response of kidney cells in unilaterally nephrectomized rats after various feeding procedures is shown in Fig. 3. In the animals which were maintained on full food throughout (Group I), labeling indices at 8-hour intervals demonstrated a rise at 24 hours which was maintained above control levels for 1 week. Food was withheld from the remaining animals (Group IV–VI) for 24 hours before nephrectomy, explaining the low number of labeled nuclei found initially. The labeling index remained below 0.1% for the entire fasting period (Group IV), and did not change with minimal quantities of food sufficient to maintain body weight (Group V). Two days after resumption of *ad libitum* feeding, values were in the normal range.

The response of kidney cells to starvation was not duplicated in cells of bone marrow. After 48 hours of starvation the labeling index of nucleated bone marrow cells was 16.4 ± 1.8% (SD) as compared to 6.9 ± 1.6% in animals with normal feeding. Variations in bone marrow differential counts could account for this increase, but there is certainly no decrease in the relative number of cells in DNA synthesis with starvation as is seen in renal cells. Thus the starvation effect is not a general phenomenon in all tissues.

DNA content of kidney. As seen in Table I, the DNA content of the kidneys of 47-day-old rats on restricted food (Group C) did not differ significantly from that of normal 27-day-old rats, (Group A-1). It was significantly lower ($p = .01$) than that of rats of the same age (Group A-3) or of younger rats (37 days) of the same weight but fed *ad libitum* (Group A-2). It was not significantly higher ($p > .05$) than the content of the single kidney in animals of similar age fed normal diets and studied 3 weeks after unilateral nephrectomy (Group B).

Discussion. The growth of the kidney appears to be tied to general body growth in

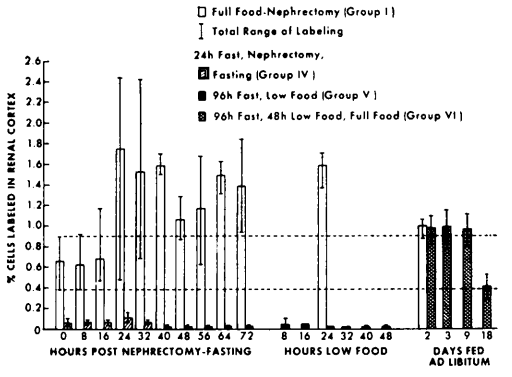

FIG. 3. Response of kidney cell labeling index in nephrectomized rats with full food, starvation, and low food intake.

TABLE I. Effect of Unilateral Nephrectomy and Food Restriction on DNA Content of Rat Kidneys.

Age (days)	No. of rats	Av body wt. (gm)	Av total kidney DNA content (mg)
A. Normal food intake; no nephrectomy—2 kidneys			
1. 27	18	85	4.36 ± .46[a]
2. 37	5	138	4.78 ± .31
3. 47	6	171	5.09 ± .33
B. Normal food intake; 3 weeks after left nephrectomy, 1 kidney			
49	21	166	3.84 ± .38
C. 3 weeks food restriction (7 gm/day); no nephrectomy—2 kidneys			
47	6	136	4.11 ± .23

[a] Standard deviation.

rats. In studies with 3- to 16-week-old female rats, Wachtel and Cole found a correlation between kidney weight, DNA content, and body weight (5). Kidney weight loss occurred, in the present study, in conjunction with starvation demonstrating that renal weight continued to follow body weight in reverse direction. However when a unilateral nephrectomy was performed in control animals, renal weight increased proportionally more than body weight, and after starvation the renal weight did not decrease with body weight. This suggests that body weight changes are not the only factor determining kidney weight.

As a result of decreased food intake, the flash labeling index in the weanling kidney (.65%) decreased markedly to levels at 24 hours (.06%) below those seen in adult rats (.11%) after normal feeding (1). The decrease was rapid with a definite effect present at 16 hours. Even the stimulus of nephrectomy did not cause an increase in labeling in the absence of adequate diet. Increase in labeling with resumption of feeding occurred after a 16-hour delay. The time relationships suggest that the response is tied to food intake and digestion and that, as soon as the gut is emptied and blood levels of metabolites such as glucose and amino acids are decreased, renal proliferation also falls to a low level. Conversely, reestablishement of proliferation may be linked to digestion of food as indicated by the 16-hour delay. Failure of bone marrow labeling to decrease with starvation suggests that the effect is a specific one for kidney and not a general effect secondary to decreased metabolic precursors.

These results and the finding in this study that prolonged food restriction at a level of 7 gm/day allowed body weight to increase from 85 to 136 gm but completely inhibited increase in renal DNA content in nonnephrectomized animals is very strong evidence that growth per se is not the only stimulus but that the level of ingested food is also important in determining kidney growth. With normal food intake the DNA content in the remaining kidney after nephrectomy increased markedly but not to the level of the content of two kidneys in an animal of similar age. This suggests that excess renal capacity exists in the normal animal with two kidneys.

Royce (10) found that both the trauma associated with ureteroperitoneostomy as well as food and water restriction and induction of peritonitis by talc instillation eliminated renal growth response. He concluded that reduced renal excretory functional capacity may be the stimulus for growth and that reduced food intake removes the stimulus even in the face of uninephrectomy.

As recently discussed by Johnson and colleagues (11, 12), the chief work of the kidney in terms of caloric utilization may be proper selection of ions for tubular reabsorption. During starvation the levels of glucose, amino acids, and various electrolytes, the basal metabolic rate, the renal plasma flow, and the glomerular filtration rate would be decreased (13,14). The increase in RNA and protein synthesis which begins 3 hours after nephrectomy (12) could be in preparation for proliferation. However the delay in onset of DNA synthesis for 12–20 hours following nephrectomy suggests that the initial response is hypertrophy of cells and that proliferation follows hypertrophy as a reaction to the need for more functional capacity.

Numerous studies have indicated that sublethal whole body irradiation causes a decrease in food intake, body weight, and growth in young animals, and a weight loss in adult animals (15). This is due chiefly to a decrease in food intake during the first week following exposure. Thus any effect of whole or partial body irradiation on kidney cell proliferation will be complicated by effects due to decreased food intake. The role of food intake suggested by previous radiation studies (5,6) is confirmed in this report.

Summary. The effect of variation in food intake and of unilateral nephrectomy on cellular proliferation in the kidney has been measured in weanling rats by means of autoradiography following flash labeling with tritiated thymidine. Labeling was used as an index of the proportion of cells engaged in DNA synthesis and thus in active proliferation. The labeling index decreased markedly from 0.65% to 0.06% after 24 hours of fasting. Refeeding after a 20-hour fast resulted in increased labeling after a 16-hour interval with return to normal levels at 24 hours. The stimulus of unilateral nephrectomy, which causes an increase in labeling index to 1.9% in animals fed *ad libitum*, failed to increase the index above 0.1% in fasting animals or in animals given 6 gm of food/day following a 4-day fast. The results suggest that renal work load may be the major factor stimulating cellular proliferation in the kidney, since loss of renal mass caused by nephrectomy failed to cause cellular proliferation in animals deprived of high levels of new metabolic products by fasting.

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Facilitation of Avoidance Conditioning by Barbiturate* with Stimulant Properties† (33069)

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Phetharbital (*N*-phenylbarbital, Pyrictal), an anticonvulsant developed for the prophylactic treatment of febrile convulsions (1), is less sedative than phenobarbital and appears to enhance mental alertness in some patients with epilepsy. The present investigation was

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