

# Macromolecular Metabolism in Compensatory Renal Hypertrophy

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1. The initial biochemical changes of compensatory hypertrophy occur well within 1 hour of unilateral nephrectomy and perhaps within the first few minutes.
  2. The initial increment in rRNA is from decreased metabolism rather than from increased synthesis.
  3. Changes in the processing of mRNA precursors are probably also important.
  4. Compensatory hypertrophy is regulated by a humoral stimulus or stimuli.
  5. The stimulus needs to be present virtually all of the time during the early phases of compensatory hypertrophy.
  6. The stimulus is related to loss of renal mass, not to loss of renal function.
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Biochemical changes of compensatory renal hypertrophy begin well within an hour from the time one kidney is removed [1]. Indeed, the work of Dicker [2] and of Lowenstein [3] suggests that the initial changes may occur within several minutes of unilateral nephrectomy. This discussion reviews the contribution of nucleic acid metabolism to the early stages of renal hypertrophy.

## ACCRETION OF RNA

Although visible growth of the mouse kidney is present within a week of unilateral nephrectomy, the major changes do not occur until the second to the fourth weeks (Fig. 1) [4]. The accretion of renal mass is true growth—an increase in dry weight, not merely an increase in the wet weight of the kidney.

Maximal accretion of RNA per cell is present, however, within 2 days of unilateral nephrectomy and holds at a plateau for nearly a month. By the second day after unilateral nephrectomy there is perhaps a 40 percent increment in the ratio of RNA to DNA in the contralateral rat kidney [5-8] and a 20 percent increase in the contralateral mouse kidney [4] (Fig. 2). RNA/DNA is a measure of cellular RNA content [5,9].

Because most RNA is ribosomal RNA (rRNA) [10,11], the amount of cellular rRNA must therefore increase within 48 hours of contralateral nephrectomy. Either an increase in rRNA synthesis or a decrease in degradation could account for the augmentation. Although early studies agreed that the half-life of ribosomal RNA after nephrectomy is unchanged [4,12] and that, by implication, the synthesis of RNA is increased, recent studies [11] (to be discussed later) require revision of this view. Immediately after nephrectomy, degradation is slowed, and only later is RNA synthesis increased.

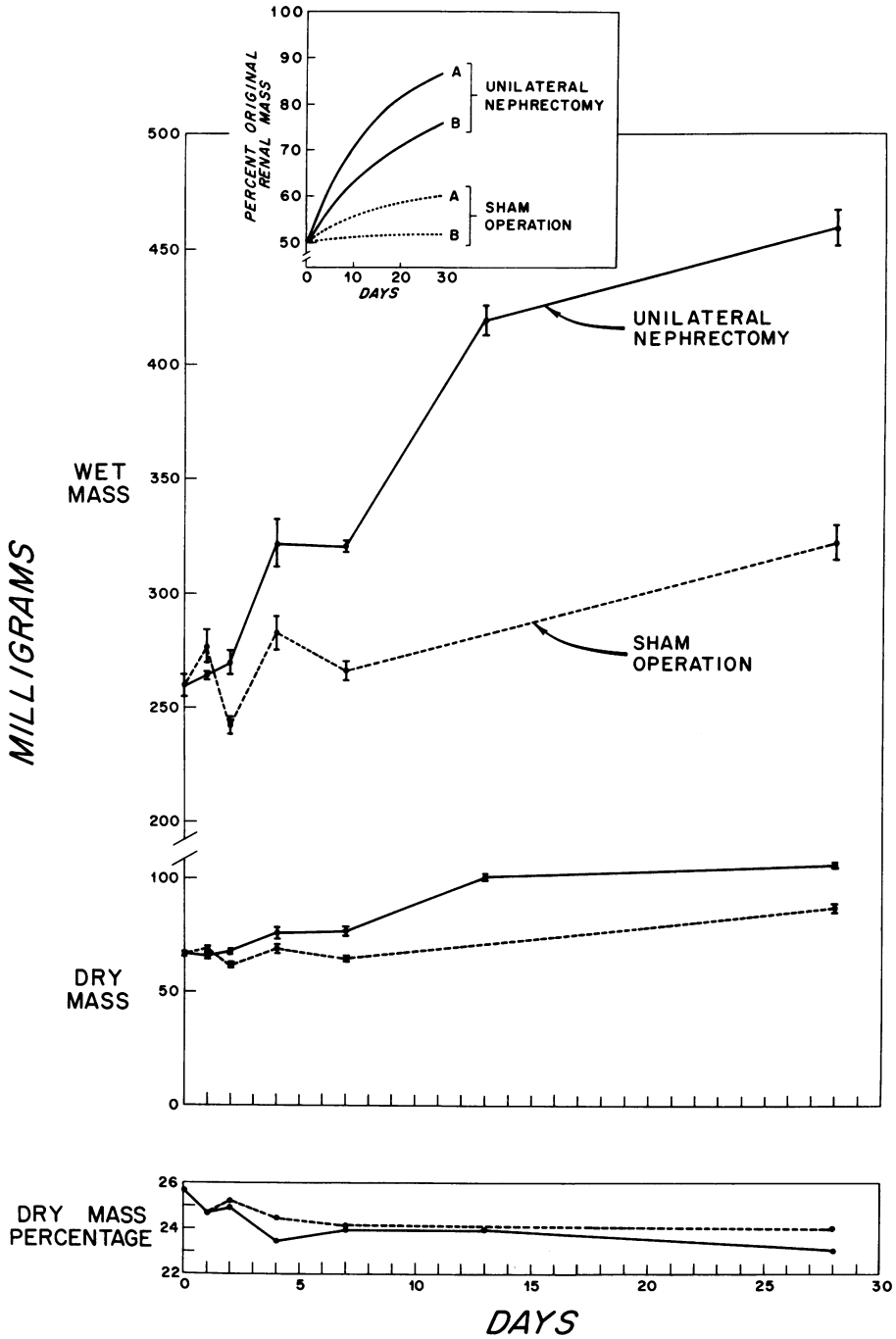


FIG. 1. Changes in mass during compensatory renal hypertrophy in mice. [By permission, Am J Physiol.]

### LOCALIZATION

Measurements of nucleic acids in the separated tubules and glomeruli disclose a 35-40 percent increase in rRNA in the renal tubules between 24 and 48 hours after unilateral nephrectomy, but no increase in the nucleic acid content of the glomeruli [13]. Moreover, when the nucleic acids of tubules and glomeruli are labelled with radioactive precursors, the extracted RNA originates exclusively from the tubules.

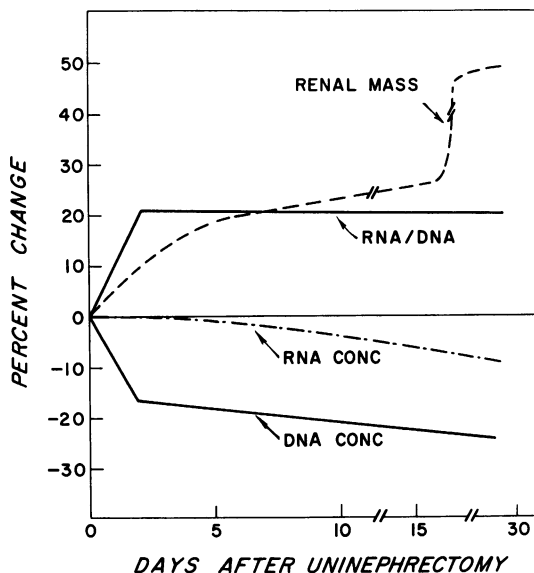


FIG. 2. Idealized pattern of comparing changes in the average cellular content of RNA (RNA/DNA) with those in renal mass. [By permission, New Eng J Med.]

The localization of labelled and extracted nucleic acids can be circumscribed still further by the choice of radioactive precursors [14]. Renal RNA is intensely labelled with orotic acid, and 97 percent of the label is localized in tubules, mostly in proximal tubules. But RNA labelled with radioactive uridine is only 80 percent in tubules, with a ratio in the proximal tubules to distal tubules of 2:1.

The apparent enlargement of glomeruli during compensatory hypertrophy is simply a result of glomerular hyperemia [1]. Engorgement of glomeruli with blood is readily visible under a dissecting microscope. When the glomeruli are separated by differential ultracentrifugation, their mean diameters are unchanged during compensatory hypertrophy, irrespective of the tonicity of the medium in which they are suspended [13].

### MITOSIS

Although mitotic activity and accretion of DNA are present during compensatory hypertrophy [15], they are not essential for the response. For example, the peak in mitotic activity identified 2 days after unilateral nephrectomy is abolished in animals treated with azathioprine, but adaptive hypertrophy is not [16].

Some experiments purporting a greater contribution from hyperplasia have to be discounted because they did not include autoradiographic confirmation of the sites of incorporation of radiolabelled thymidine into DNA. Minor degrees of trauma to a kidney promote subcapsular and otherwise ectopic localization of radioactive thymidine in DNA without an increase in the tubular rate of DNA synthesis (Fig. 3) [17]. This adventitial uptake of thymidine can be many times the modest, normal hyperplastic response.

### RIBOSOMAL RNA METABOLISM

In cultured mammalian cells rRNA originates from a nucleolar precursor considerably larger than the mature rRNA in the cytoplasm [18]. The progenitive molecule is a 45S precursor, from which portions are sequentially clipped and exported from nucleus to cytoplasm.

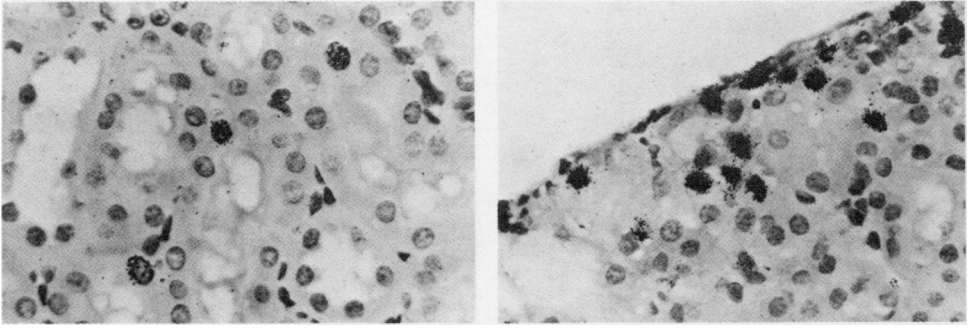


FIG. 3. Autoradiograph showing uptake of  $^3\text{H}$ -thymidine in tubule cells during compensatory renal hypertrophy (A) contrasted to uptake in subcapsular cells produced by minor trauma (B). [By permission, Proc Soc Exper Biol Med.]

Nucleoli from kidney suitable for extraction of undegraded rRNA precursors may be prepared by sieving kidneys through 44 stainless steel screens, followed by a rapid washing to remove residual cytoplasmic elements [19]. Electropherograms of the extracted nucleolar nucleic acids demonstrate the same species of precursor and intermediary forms of rRNA as in cultured cells, the sole exception being a somewhat greater amount of 36S RNA in kidney (Fig. 4).

The number of precursor molecules is different, however. The renal cell contains only 400 to 600 45S RNA precursor molecules, in contrast to perhaps 10,000 45S RNA precursors in cultured cells [19]. The relatively few renal rRNA precursors turn

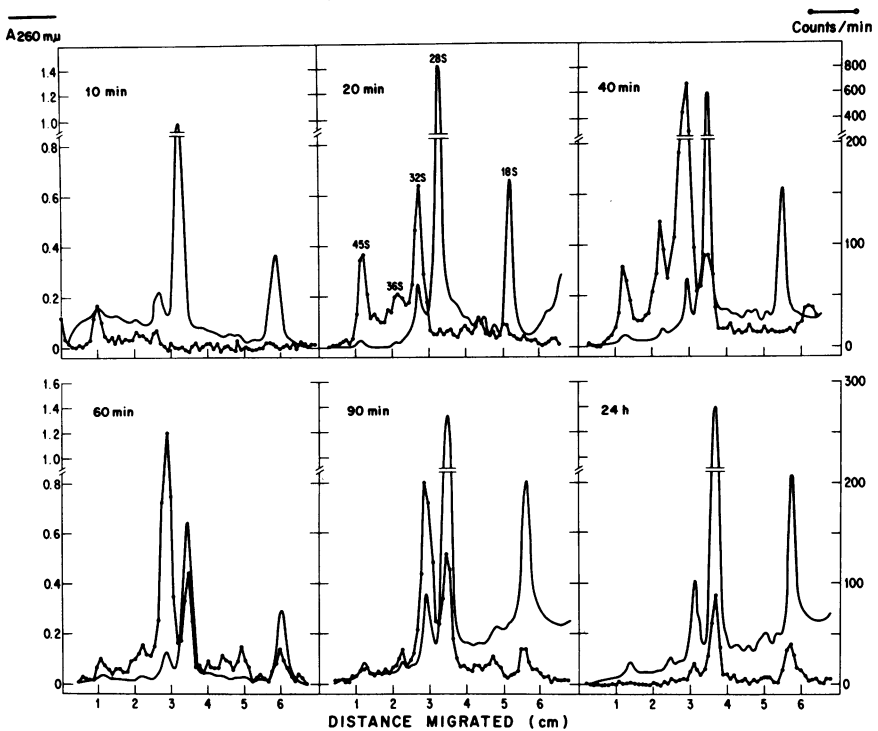


FIG. 4. Processing of methionine-labelled nucleolar RNA in normal mouse kidney. Labelling times are shown. [By permission, J Cell Biol.]

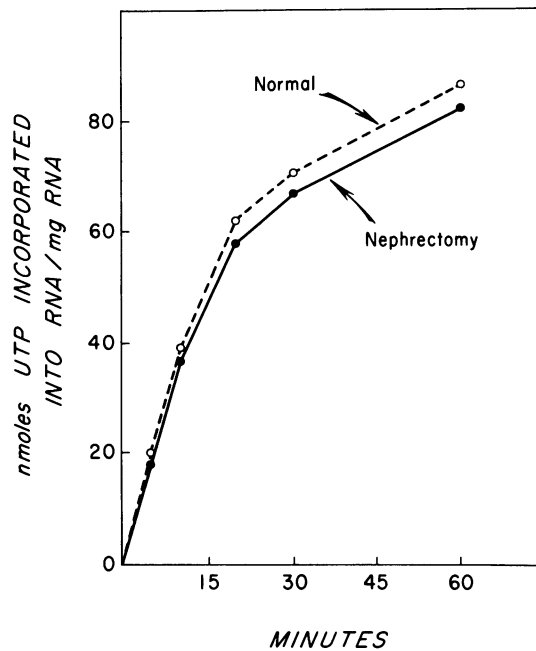


FIG. 5. Unchanged rate of RNA synthesis in early stages of compensatory renal hypertrophy after labelling with  $^3\text{H}$ -uridine. [By permission, *Biochem J.*]

over with the half-life of only 4 to 5 minutes as compared with a half-life perhaps five to ten times that long in the cultured cells. Since there are so few molecules turning over so rapidly, the puzzle is how they could speed up their activity to account for the accretion of ribosomal RNA during compensatory renal hypertrophy. Is processing speeded still further, or do more precursors become available?

Calculations of actual rates of RNA synthesis from amounts of the immediate precursor (UTP) entering RNA show no change in the rate of rRNA synthesis immediately after the onset of compensatory hypertrophy (Fig. 5) [20]. Discrepancy between these observations and those to the contrary recently reported [21] are not readily resolved.

If RNA synthesis is not increased and if earlier studies showing unchanged half-life during compensatory hypertrophy are correct, an explanation of the accretion of RNA seems an insoluble paradox. Reassessment of RNA turnover times by contemporary techniques, however, does identify a change in half-life during the initial stages of compensatory hypertrophy (Fig. 6).

The normal turnover time of 18S and 28S RNA is slowed at first. Measurement of turnover in parallel with isotope dilution studies to ascertain recovery shows that all the increment in RNA found during the first 2 days of compensatory hypertrophy is explicable by a slower rate of degradation of rRNA (Fig. 7). Most additional RNA during the first 4 days, in fact, can be accounted for by a change in the rate of degradation rather than by an increase in synthetic rate. However, at 4 days, an increase in the rate of synthesis of rRNA is sufficient to account for the increased cellular content of RNA. Precedents for regulation by degradation rather than by synthesis are the conservation of protein in liver during starvation and in compensatory hyperplasia of the liver [22] and in salivary glands during isoproterenol-

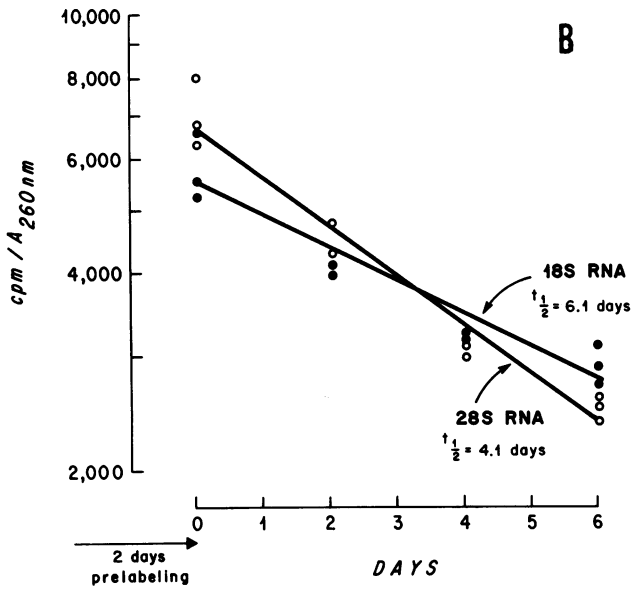


FIG. 6. Degradation of 18 S and 28 S RNA in normal kidney after prelabelling. [By permission, J Cell Biol.]

stimulated growth [23] and the conservation of RNA in cortisone-stimulated liver [24].

### MESSENGER RNA METABOLISM

Besides the contribution of changes in rRNA metabolism to the genesis of compensatory hypertrophy, messenger RNA (mRNA) must also be involved, as Ouellette [25] explained in detail. There is good reason to think that mRNA metabolism is affected soon after hypertrophy.

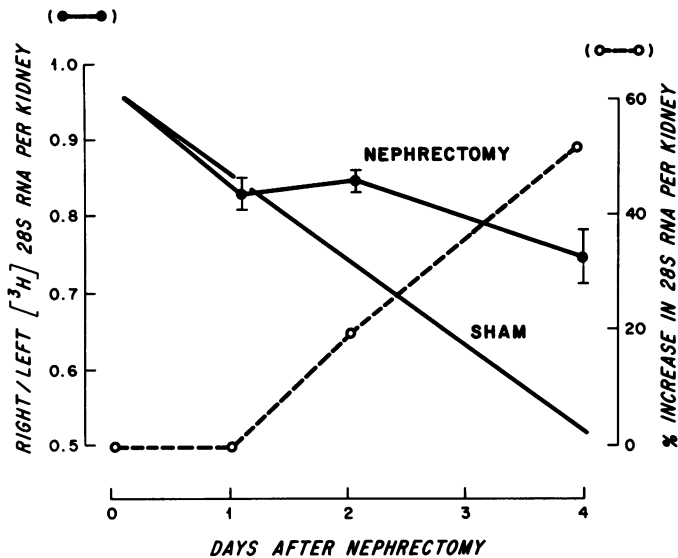


FIG. 7. Slower degradation of 28 S RNA in the early stages of compensatory renal hypertrophy compared with the increase in 28 S RNA.

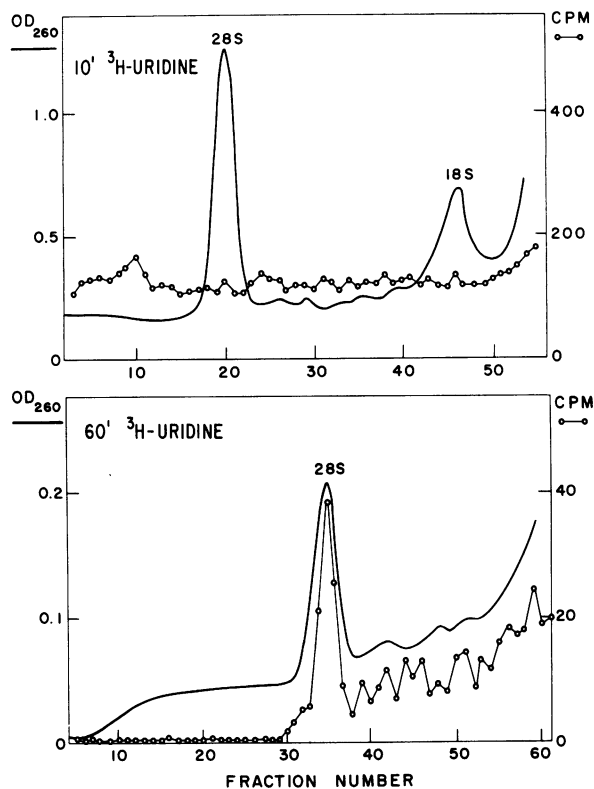


FIG. 8. Processing of hnRNA labelled with  $^3\text{H}$ -uridine after contralateral nephrectomy. [By permission, Proc Nat Acad Sci.] Labelling was begun after nephrectomy and terminated after 10 minutes or 60 minutes. Similar results were obtained when uridine of greater specific activity is used.

mRNA seems to originate from the nucleoplasm, where some or all of it is formed from large precursor molecules of heterodisperse sedimentation characteristics (hnRNA). Processing of hnRNA is accelerated within 1 hour of contralateral nephrectomy [26]; hnRNA abundant in the remaining kidney 10 minutes after unilateral nephrectomy is absent 60 minutes later (Fig. 8). Perhaps these early changes in hnRNA metabolism, those of cyclic nucleotide [1,27] and those of phospholipid synthesis [2] will all ultimately point to the central events activated by unilateral nephrectomy.

### STIMULI

Because a heterotopically transplanted kidney will undergo compensatory hypertrophy, the stimulators of compensatory hypertrophy are by inference blood-borne [28]. Direct confirmation comes from studies in which rats were cross-circulated by a technique of vascular parabiosis that permits exchange of approximately 10 to 15 percent of the blood volume per minute [29,30]. Removal of 2 kidneys from one animal produces increments in renal mass and in RNA/DNA of the other animal precisely those resulting from loss of 50 percent of the renal mass in a single rat (Fig. 9). Removal of 3 kidneys between the two animals provokes a serially greater increase in mass and in RNA/DNA. If 3 kidneys are removed between two animals cross-circulated for 72 hours or if 1 kidney supplies three animals linked in vascular parabiosis, there is an intense mitotic activity in the remaining kidney.

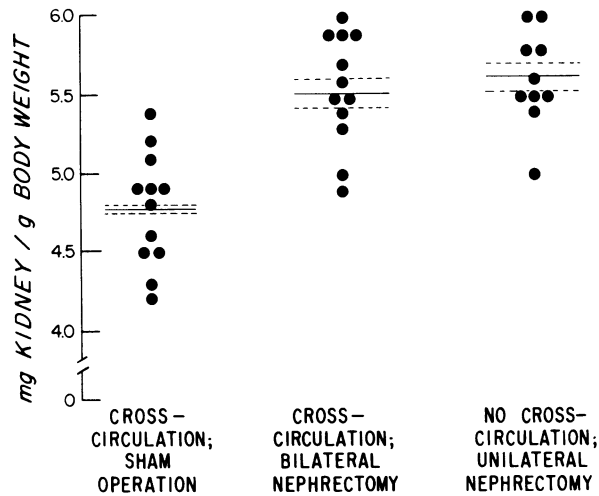


FIG. 9. Humoral stimulation of renal mass after 48 hours of vascular parabiosis with an anephric rat compared with unilateral nephrectomy in a single rat. [By permission, Surgery.]

Whatever the blood-borne mediators of compensatory hypertrophy are, if hypertrophy is to persist they need to be present nearly all the time during the early stages [30]. When compensatory hypertrophy is produced in the 2 kidneys of one animal by cross-circulation with an anephric donor, interruption of the cross-circulation is responsible for a return in RNA/DNA to normal levels with 6 hours after disconnection and in renal weight to normal within 12 hours (Fig. 10). Compensatory renal hypertrophy is, therefore, not a fixed phenomenon, but is plastic, subject to homeostatic needs.

There is more evidence about what the humoral mediators are not than about what they are. They are not factors under control of the endocrine system regulated by the hypophysis [31,32] or under the influence of pancreatic endocrine hormones [33]. They are not dependent on the presence of any of the abdominal viscera, with the possible exception of the liver [34]. They are independent of loss of ability to excrete

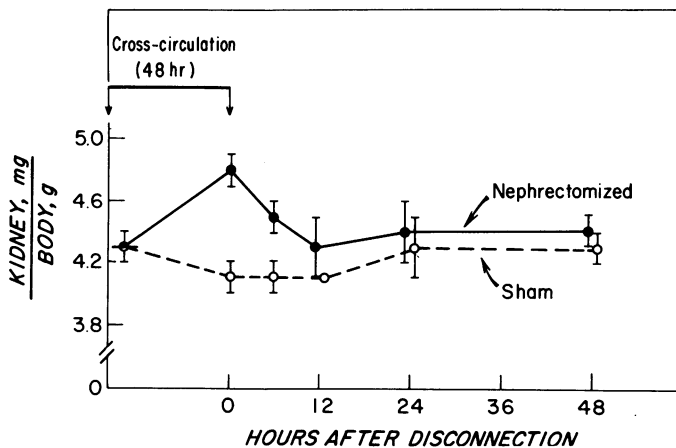


FIG. 10. Rate of decline in renal mass after removal of the humoral stimulus transmitted by cross-circulation. [By permission, Surgery.]



as a consequence of unilateral ureteral ligation [35]. They are not substances present in the renal vein and inactivated by the liver [36]. They are not influenced by fourfold increases in the serum levels of creatinine [35]. At the moment it is quite impossible to distinguish whether compensatory hypertrophy is regulated by removal of an inhibitory substance or by addition of a stimulating factor. My hypothesis (without a shred of evidence to demonstrate its truth) is that loss of renal mass activates transformation of an inactive precursor stimulating substance into its active form. The concept of "work hypertrophy" is passé [28].

#### ACKNOWLEDGEMENTS

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#### REFERENCES

1. Bucher NLR, Malt RA: Regeneration of liver and kidney. Boston, Little, Brown and Company, 1971
2. Dicker SE: This issue
3. Lowenstein L: This issue
4. Malt RA, Lemaitre DA: Accretion and turnover of RNA in the renoprival kidney. *Am J Physiol* 214:1041-1047, 1968
5. Halliburton IW, Thomson RY: Chemical aspects of compensatory renal hypertrophy. *Cancer Res* 25:1882-1887, 1965
6. Threlfall G, Taylor DM, Buck AT: Studies of the changes in growth and DNA synthesis in the rat kidney during experimentally induced renal hypertrophy. *Am J Pathol* 50:1-14, 1967
7. Kurnick NB, Lindsay PA: Nucleic acids in compensatory renal hypertrophy. *Lab Invest* 18:700-708, 1968
8. Dicker SE, Shirley DG: Mechanism of compensatory renal hypertrophy. *J Physiol* 219:507-523, 1971
9. Vendrely R: The deoxyribonucleic acid content of the nucleus. In: *The Nucleic Acids*, Chargaff E, Davidson JN, eds. New York: Academic, 1955, vol II, pp 155-180
10. Hirsch CA: Quantitative determination of the ribosomal ribonucleic acid content of liver and Novikoff hepatoma from fed and from fasted rats. *J Biol Chem* 242:2822-2827, 1967
11. Melvin WT, Kumar A, Malt RA: Conservation of ribosomal RNA during compensatory renal hypertrophy: a major mechanism in RNA accretion. *J Cell Biol* 69:548-556, 1976
12. Avdalović N: Disappearance of radioactivity from the various ribonucleic acid pools and acid-soluble fractions of mouse liver and kidney after a single injection of labelled orotic acid: the effect of castration. *Biochem J* 119:331-338, 1970
13. Vančura P, Miller WL, Little JW, Malt RA: Contribution of glomerular and tubular RNA synthesis to compensatory renal growth. *Am J Physiol* 219:78-83, 1970
14. Ross JS, Malamud D, Caulfield JB, Malt RA: Differential labeling with orotic acid and uridine in compensatory renal hypertrophy. *Am J Physiol* 229:952-954, 1975
15. Nowinski WW, Goss RJ, eds: *Compensatory renal hypertrophy*. New York: Academic Press, 1969
16. Cobbe SM, Herbertson BM, Houghton JB: Some effects of azathioprine on compensatory renal enlargement in mice. *Transplantation* 10: 443-446, 1970
17. Malamud D, Paddock J, Malt RA: Mitosis and DNA synthesis in mouse kidney: sources of error in evaluating cell proliferation. *Proc Soc Exp Biol Med* 139:28-31, 1972
18. Perry RP: Processing of RNA. *Ann Rev Biochem* 45:605-629, 1976
19. AB G, Malt RA: Metabolism of ribosomal precursor RNA in kidney. *J Cell Biol* 46:362-369, 1970
20. Hill JM, AB G, Malt RA: Ribonucleic acid labelling and nucleotide pools during compensatory renal hypertrophy. *Biochem J* 144:447-453, 1974
21. Cortes P, Levin NW, Martin PR: Ribonucleic acid synthesis in the renal cortex at the initiation of compensatory growth. *Biochem J* 158:457-470, 1976
22. Conde RD, Scornik OA: Role of protein degradation in the growth of livers after a nutritional shift. *Biochem J* 158:385-390, 1976
23. Hill JM, Malamud D: Decreased protein catabolism during stimulated growth. *FEBS Letters* 46:308-311, 1974
24. Ottolenghi C, Barnabel O: Reduced breakdown *in vivo* of liver microsomal ribonucleic acid and protein in rats treated with cortisone. *Endocrinology* 86:949-954, 1970
25. Ouellette AJ: This issue
26. Willems M, Musilova HA, Malt RA: Giant nucleoplasmic RNA in the switch on of compensatory renal growth. *Proc Nat Acad Sci* 62:1189-1194, 1969

27. Schlondorff D, Weber H: Cyclic nucleotide metabolism in compensatory renal hypertrophy and neonatal kidney growth. *Proc Nat Acad Sci* 73:524-528, 1976
28. Malt RA: Renal growth factor. In: *Humoral control of growth and differentiation: vertebrate regulatory factors*, LoBue J, Gordon AS, eds. New York: Academic Press, 1973, vol 1, pp 257-273
29. Van Vroonhoven TJ, Soler-Montesinos L, Malt RA: Humoral regulation of renal mass. *Surgery* 72:300-305, 1972
30. Dijkhuis CM, van Urk H, Malamud D, Malt RA: Rapid reversal of compensatory renal hypertrophy after withdrawal of the stimulus. *Surgery* 78:476-480, 1975
31. Reiter RJ: The endocrines and compensatory renal enlargement, In: *Compensatory Renal Hypertrophy*, Nowinski WW, Goss RJ, eds. New York: Academic Press, 1969, vol 25, p 493
32. Ross J, Goldman JK: Compensatory renal hypertrophy in hypophysectomized rats. *Endocrinology* 87:620-624, 1970
33. Ross J, Goldman JK: Effect of streptozotocin-induced diabetes on kidney weight and compensatory hypertrophy. *Endocrinology* 88:1079-1082, 1970
34. Ross JS, Bucher NLR, Malt RA: Compensatory renal hypertrophy in eviscerated rats. *Cancer Res* 34:502-505, 1974
35. Obertop H, Malt RA: Lost mass and excretion as stimuli to parabiotic compensatory renal hypertrophy. *Am J Physiol* (in press)
36. Bump S, Malt RA: Renomesenteric venous shunt in the rat: effect on size and nucleic acid content of the kidneys. *J Appl Physiol* 28:225-226, 1970