Increased Aspartate Carbamyltransferase Activity in Compensatory Renal Growth[1] (36371)

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Unilateral nephrectomy leads to the compensatory growth of the remaining kidney and to enhanced activity of biochemical parameters associated with cellular growth. Within hours there is increased uptake of radiolabeled nucleotides and synthesis of RNA and protein with stimulation of DNA synthesis occurring somewhat later (1–7). Induction of certain enzymes involved in DNA synthesis is maximal in 24–48 hr (8). This is a report of renal aspartate carbamyltransferase (ACTase) in the uninephrectomized rat (9). ACTase (EC2.1.3.2) catalyzes the first step unique to pyrimidine biosynthesis, the carbamylation of aspartate by carbamyl phosphate (10). The product, carbamyl aspartate, is a precursor of orotic acid; therefore, of the pyrimidines and ultimately of RNA and DNA. In bacteria the control of ACTase by nucleotides is a classical example of biochemical feedback (11). ACTase is particularly active in plant and animal tissues which are either growing rapidly or undergoing constant renewal, *e.g.*, apical meristems, plant root tips, regenerating liver, fetal tissues, intestinal mucosa, and testis. Unusually high ACTase activities occur in certain tumors (10).

Materials and Methods. Female Wistar rats, 150–200 g, were kept in groups of four or five in stainless-steel cages containing corn cob bedding. They had free access to Purina Laboratory Chow and water. Nephrectomies were performed under ether anesthesia. After ligating its blood vessel connections, the right and larger kidney was removed through dorsal, subcostal skin, and muscle incisions. In some experiments ACTase activity of this kidney was assayed. Control animals were sham operated. At various times after uninephrectomy all rats were killed by decapitation. After perfusion with isotonic NaCl via the abdominal aorta the remaining kidney was removed, blotted, and weighed. A 1:15 (w/v) homogenate in ice-cold isotonic KCl was prepared and centrifuged (29,000*g*) for 1 hr at 4°. After removing the surface layer of fat, ACTase in the supernatant was assayed by Lowenstein and Cohen's method (12) modified for liquid-scintillation counting (9). The reaction:

$$^{14}\text{C carbamyl phosphate} + \text{aspartate} \xrightarrow{\text{ACTase}} {}^{14}\text{C carbamyl aspartate} + P_i,$$

was conducted in 12-ml conical centrifuge tubes for 20 min at 38°. The medium consisted of diethanolamine (DEA) buffer 0.1 *M* (pH 9.2), K aspartate 10 m*M*, ^{14}C carbamyl phosphate 2 m*M*, and 0.5 ml supernatant in a total volume of 1 ml. Under these conditions the carbamyl aspartate counts per minute were linearly related to enzyme concentration. The reaction was terminated by adding 0.5 ml 1 *N* perchloric acid which also decomposed the residual acid labile carbamyl phosphate. The $^{14}CO_2$ (and CO_2) resulting from this decomposition were driven off by shaking for 20 min in a water bath at 80°. The reaction tubes were subsequently centrifuged for 10 min at 900*g* and 0.5 ml of supernatant was transferred to counting vials containing 10 ml Bray's (13) solution. The samples were counted for 10 min in a Packard liquid-scintillation spectrometer (Model 3310). In this system the quench was relatively constant from sample to sample and the efficiency was between 50 and

[1] Supported by a grant from the National Institute of Arthritis and Metabolic Diseases.

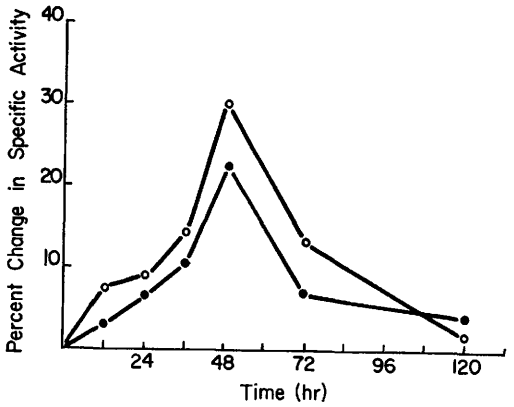

FIG. 1. Renal aspartate carbamyltransferase activity after unilateral nephrectomy expressed as the percentage of change from control values. O—O carbamyl aspartate counts per hour per gram kidney; ●—● carbamyl aspartate counts per hour per milligram supernatant N.

60%. Supernatant nitrogen (N) content was routinely estimated by a modification of the Nessler method. The results are expressed as specific activity per gram of tissue and specific activity per milligram N where specific activity is defined as carbamyl aspartate counts per hour.

Results and Discussion. Figure 1 shows the time course of changes in renal ACTase for 5 days after unilateral nephrectomy. Carbamyl aspartate specific activity based on wet weight and on N peaked at 48 hr. Thereafter it fell, dropping to normal values by day 5. The maximal increase in N concentration occurred between 48 and 72 hr. This increase in N is not due to a proportional change in the dry weight of the hypertrophying kidney (% dry weight sham-operated vs. uninephrectomized: 22.9 ± 0.16 SE vs. 23.3 ± 0.32 SE $p > .05$). During the 5 days, the weight of the remaining kidney increased progressively. However, comparing the left kidney weight of operated animals with that in sham-operated controls proved somewhat unsatisfactory as an index of growth because in the rat the kidney weight is not necessarily proportional to body weight. In subsequent experiments we assessed renal growth by comparing the ratio of left to right kidney weight in experimental and control rats. The assumption was made that in 48 hr the normal growth of the right (removed) kidney is negligible relative to that in the compensating kidney. Our value, 0.94 ± .02 SE, for intact rats agrees with Williams' ratio of 0.9 (14). Forty-eight hours after uninephrectomy this ratio increased by 22% (1.15 ± .02 SE, $p < .001$). During this period average body weight gain in the experimental and control groups was 2 and 6 g, respectively.

The major contribution to the renal growth which follows uninephrectomy is the hypertrophy and hyperplasia of the proximal convoluted tubules (15, 16). The possibility existed that if the ACTase activity in the renal cortex were normally higher than in the medulla the relatively greater increase in cortical mass *per se* could account for the observed rise in enzyme activity.

Forty-eight hours after uninephrectomy

TABLE I. Renal Cortical Aspartate Carbamyltransferase Activity 48 hr After Uninephrectomy Compared with That in the Renal Cortex and Intact Kidneys of Sham-operated Controls.

	N	Specific activity[a]/g kidney	Supernatant N (mg/g kidney)	Specific activity/mg N	Left:right kidney ratio
Intact kidney (sham)	7	1501 ± 50.1[b]	12.9 ± 0.4	117.1 ± 4.3	0.94 ± 0.02
Renal cortex (sham)	7	1748 ± 26.7[c]	14.1 ± 0.3[d]	126.2 ± 4.6[e]	
Renal cortex (nephrect.)	8	2028 ± 52.9[c]	14.6 ± 0.2[c]	138.7 ± 3.2[d]	1.15 ± 0.02[f]

[a] Specific activity = acid-stable carbamyl aspartate counts/hr (1.4×10^6 cpm = 2 μmol carbamyl phosphate).
[b] Mean ± SE.
[c] $p < .01$.
[d] $p < .05$.
[e] Not significant.
[f] $p < .001$.

the remaining left kidneys were removed, weighed, and bisected sagittally. The medullary tissue was scraped from the cortex and cortical ACTase determined. The left kidney from sham-operated control rats was treated identically while the intact right kidney was also assayed. Table I shows that in control animals renal cortical ACTase activity is significantly higher than in the intact kidney. But because cortical N concentration is also higher, ACTase activity based on N does not differ significantly. In the hypertrophying kidney cortical N concentration, cortical ACTase activity, and left:right kidney weight ratios are all significantly elevated. Thus, both the greater cortical mass and enhanced enzyme activity contribute to the early increase in ACTase activity in compensatory renal growth.

1. Simpson, D. P., Amer. J. Physiol. **201**, 517 and 523 (1961).

2. Halliburton, I. W., and Thompson, R. Y., Cancer Res. **25**, 1882 (1965).

3. Johnson, H. A., and Vera Roman, J. M., Amer. J. Pathol. **49**, 1 (1966).

4. Threlfall, G., Taylor, D. M., and Buck, A. T., Amer. J. Pathol. **50**, 1 (1967).

5. Malt, R. A., and LeMaitre, D. A., Amer. J. Physiol. **214**, 1041 (1968).

6. Reiter, R. J., Lab. Invest. **14**, 1636 (1965).

7. Reiter, R. J., Comp. Biochem. Physiol. **25**, 493 (1968).

8. Mayfield, E. D., Jr., Liebelt, R. A., and Bresnick, E., Cancer Res. **27**, 1652 (1967).

9. Waymire, J. C., Studies on Renal Aspartate Carbamyltransferase, Ph.D. Dissertation, Ohio State Univ. (1969).

10. Cohen, P. P., and Brown, G. W., Jr., *in* "Comparative Biochemistry" (M. Florkin and H. S. Mason, eds.), Vol. 2, p. 226. Academic Press, New York (1960).

11. Yates, R. A., and Pardee, A. P., J. Biol. Chem. **221**, 757 (1956).

12. Lowenstein, J. M., and Cohen, P. P., J. Amer. Chem. Soc. **76**, 5571 (1954).

13. Bray, G., Anal. Biochem. **1**, 279 (1960).

14. Williams, G. E., Lab. Invest. **11**, 1295 (1962).

15. Rollanson, H. D., Anat. Rec. **104**, 263 (1949).

16. McCreight, C. E., and Sulkin, N., J. Gerontol. **14**, 440 (1959).

Received Dec. 29, 1971. P.S.E.B.M., 1972, Vol. 139.