Hexose Monophosphate Shunt Activity in Compensatory Renal Hypertrophy* (33430)

JAMES K. FARQUHAR, WALTER N. SCOTT,[1] AND FREDRIC L. COE[2]
(Introduced by W. A. Brodsky)
Physiological Chemistry Section, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas

After either unilateral nephrectomy or bilateral heminephrectomy, there is a rapid increase in the size and the protein content of remaining kidney tissue due both to cellular hypertrophy and to hyperplasia (1). Although it has recently been demonstrated that there is a rapid increase in the rate of protein synthesis concomitant with the increase in protein content of the residual kidney tissue (2), there is only a small amount of data describing the response of specific renal enzyme systems in this surge of growth (3).

Glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) are "controlling" enzymes for the oxidative limb of the pentose cycle. This cycle is responsible for the synthesis of all (4) or most (5) of the ribose required for synthesis of RNA and DNA. Although the activities of G6PD and of the hexose monophosphate shunt (HMPS) have been examined in a number of systems during periods of growth, the data offer conflicting views on the role of the HMPS in growing tissues. Human liver cell cultures evidence doubling of G6PD activity within 6 days of the onset of a growth cycle (6), and increased HMPS activity has been observed during the repair of infarcted cardiac muscle (7). Dies and Lotspeich examined renal hexose monophosphate shunt activity in rats subjected to unilateral nephrectomy and concluded that there was no increase in the HMPS dehydrogenase activity of the remaining kidney (8). Weber and Cantero as well as Ostanenko found no increase in G6PD specific activity in regenerating liver tissue *in vivo* (9). Because of the pivotal role of this enzyme system, we felt an examination of the capacity of the HMPS dehydrogenase activity in the hypertrophying kidney would contribute toward an understanding of the basic processes of tissue regeneration.

Materials and Methods. Sprague-Dawley rats were obtained from the Berkeley-Pacific Co. and maintained on an *ad libitum* diet of laboratory chow and water. Fifty-four male rats (weight 80–140 g) were designated for study at 0, 6, 12, 18, 24, 36, 48, 72, or 96 hr after left nephrectomy; the animals were each examined in a sequence determined by consulting a table of random numbers (10). To exclude the effects of any diurnal variations in renal enzyme activity, approximately equal numbers of the rats underwent the initial unilateral nephrectomy at 8 AM, 2 PM, and 8 PM in a manner determined by the original randomization of the animals. The rats were anesthetized with pentobarbital and the left kidney removed through a flank incision after ligation of the renal pedicle. After elapse of the designated period after the left nephrectomy, the right kidney was removed in the same way except that the renal pedicle was not ligated. In the nine animals studied at "O" time, the right kidney was removed immediately after excision of the left.

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[1] Current address: Institute for Medical Research and Studies and The Mount Sinai Graduate School of The City University of New York, New York, New York.

[2] Current address: Department of Medicine, University of Texas, Southwestern Medical School, Dallas, Texas.

TABLE I. Ratios (Right Kidney/Left Kidney) of Renal HMPS Dehydrogenase Activity, Total Soluble Protein, and Specific Activity.[a]

Hours		Total dehydrogenase activity R/L Mean	SE	Total soluble protein R/L Mean	SE	Dehydrogenase specific activity R/L Mean	SE
0	(9)	1.10	.09	1.05	.06	1.03	.03
6	(3)	.99	.08	.87	.02	1.02	.10
12	(6)	1.01	.06	1.00	.05	1.01	.02
18	(6)	1.17	.12	1.10	.05	1.07	.09
24	(6)	1.20	.10	1.10	.06	1.11	.09
36	(6)	1.51	.19[b]	1.19	.04	1.26	.12[b]
48	(6)	1.37	.16	1.30	.07[b]	1.05	.09
72	(6)	1.97	.23[c]	1.39	.07[c]	1.41	.10[c]
96	(6)	1.97	.12[c]	1.58	.10[d]	1.26	.08[d]

[a] The time lapse between removal of the left kidney and subsequent removal of the right kidney is indicated in Column 1. The number of animals studied in each group appears in parentheses. Probability levels comparing each postnephrectomy period with the "O" control by Student's 't' test are: [b] $p < .05$; [c] $p < .01$; [d] $p < .001$; all others NS.

Each kidney was decapsulated, weighed, and homogenized with a Ten-Broeck glass homogenizer in 7–8 ml of a buffer (11) composed of magnesium chloride 0.01 *M*, potassium phosphate (pH 7.8) 0.02 *M*, potassium bicarbonate 0.035 *M*, potassium chloride 0.025 *M*, and sucrose 0.35 *M*. The tissue homogenate was centrifuged at 700 *g* for 15 min, and this supernate at 20,000 *g* for 30 min. The resultant soluble fraction was brought to 10 ml with additional buffer and assayed for enzyme activity; the method used was a modification of that of Kornberg and Horecker (12), employing glucose-6-phosphate (10^{-3} *M*) as substrate and nicotinamide adenine dinucleotide phosphate (NADP) (5.6×10^{-4} *M*) as the hydrogen acceptor. NADP reduction was measured at 340 mμ in a Beckman DU monochromator fitted with a Gilford Model 220 absorbance indicator and recorder. The cuvette chamber was water jacketted and the temperature was maintained at 25 ± 0.5° with a constant-temperature water bath.

An aliquot of the tissue supernate was added to 5 vol of cold trichloracetic acid, centrifuged, and the recovered precipitate dissolved in 0.1 *N* NaOH. The protein content was determined by an adaptation of the biuret method on an automatic chemical analyzer.

Each animal was used as its own control. To normalize the data for variations in rat size, the total enzyme activity, protein content, and specific activity are expressed as the ratio of regenerating (right) to control (left) kidney measurements.

The data were analyzed statistically by means of the standard error of the mean and by the Student 't' test.

Results. The mean of the ratios in each group of animals is given in Table I along with its standard error. The ratio of the total HMPS dehydrogenase activity is slightly greater than 1.0 at "O" time, due, at least in part, to the known greater size (1) and protein content (2) of the right kidney of the rat. The ratio begins to increase at 18 hr and by 36 hr the dehydrogenase activity in the residual kidney is increased by about 40% ($p < .05$). A slight fall occurs at 48 hr and this is followed by a subsequent rise so that enzyme activity is nearly doubled (1.97-fold) at 72 and 96 hr ($p < .01$).

The ratio of the total "soluble" protein in the 20,000 *g* supernate is 1.05 at "O" time, which agrees well with the value of 1.06 reported for the ratio of the total kidney pro-

tein of the rat (2). After a transient fluctuation at 6 hr, the protein ratio shows a steady increase throughout the remainder of the study (Table 1); there is a 25% increase at 48 hr ($p<.05$) and a 50% increase by 96 hr ($p<.001$).

Dehydrogenase specific activity for each kidney was calculated from the total enzyme activity and the amount of soluble protein. The ratio of the specific activities was then determined for each animal as before. The table illustrates that this value begins to increase at about 18 hr, and achieves a 25% increment at 36 hr ($p<.05$). The ratio then falls to approximately 1.0 at 48 hr, subsequently rises to 1.41 at 72 hr, and falls again to 1.26 by the 96-hr period.

Discussion. The present study demonstrates that renal HMPS dehydrogenase activity increases progressively during the compensatory hypertrophy that follows unilateral nephrectomy. The magnitude of the increment in dehydrogenase activity is such that the total renal dehydrogenase is completely replenished within 72 hr; the data also suggest that achievement of the original renal dehydrogenase level is not followed by any further increase, but studies over a longer period of time will be required to confirm this observation. There are several possible explanations for the disparity between our conclusions and those of Dies and Lotspeich (8); (1) the rats in our study were younger; it has been shown repeatedly that the regenerative response to either unilateral nephrectomy (14) or partial hepatectomy (15) declines with increasing age; (2) the observations of Dies and Lotspeich were evidently made only at 24-hr intervals, and therefore they would not have observed the large increase in specific activity at 36 hr; (3) their data were expressed as enzyme activity per gram net weight rather than per gram of tissue protein, and the increased water content that accompanies compensatory hypertrophy would have obscured to some extent an increase in enzyme activity. Since the enzyme activities were determined by the same method in the two studies, the differences between the two sets of data are perhaps more related to differences in experimental technique than to a fundamental incompatibility, and this conclusion is supported to some extent by their own data that reveal a 30% increase in total HMPS dehydrogenase activity at 7 days.

The finding of substantial increases in the "soluble" protein content of the kidney was not unexpected, and the rate and magnitude of the increment correspond to those described for the total renal protein in the hypertrophying kidney.

During the period of intense growth immediately after unilateral nephrectomy, one might expect that those enzymes most essential for tissue growth, or for coping with the increased excretory load thrust upon the remaining kidney tissue, would be synthesized in preference to the large bulk of protein; this preferential synthesis would lead to a relative increase in the amount of enzyme per gram tissue protein, and would be reflected by an increased enzymatic specific activity. We have determined the HMPS dehydrogenase specific activity in the hypertrophying kidney and have found wide fluctuations in this value during the 96-hr period encompassed by our study. We found prominent peaks of specific activity at 36 and 72 hr, indicating that at these intervals after the initial nephrectomy the dehydrogenase enzymes have been synthesized at a significantly higher rate than has the remainder of the tissue proteins. It should be noted that the interval required for the specific activities to reach each of the peaks from the preceding trough is approximately the same, that the levels achieved in each instance are approximately equal, and that these peaks of specific activity are coincident with the bursts of DNA synthesis that have been described 36 and 72 hr after unilateral nephrectomy in rats of this size (14). Furthermore, this biphasic pattern of specific activity bears a striking resemblance to the pattern described for the synthetic rate of total renal protein in compensatory renal hypertrophy (2). These facts suggest that the augmented activity may be related to the increased ribose requirements attendant to accelerated cell division, whereas the sustained over-all in-

creases in total enzyme activity simply reflect balanced tissue growth.

Summary. Hexose monophosphate shunt (HMPS) dehydrogenase activity was measured in the remaining kidney at frequent intervals for 4 days after unilateral nephrectomy of rats. Total HMPS dehydrogenase activity in the residual kidney began to increase 18 hr after nephrectomy and was doubled (1.97) at 72 hr, where it remained at 96 hr (1.97). While the total soluble protein (20,000 *g* supernate) showed a continuous increase beginning about 18 hr after nephrectomy, HMPS dehydrogenase specific activity exhibited a biphasic pattern with peaks at 36 hr (25% increment) and 72 hr (40% increment). The data indicate that preferential synthesis of the HMPS dehydrogenase enzymes (G6PD and 6PGD) is an early response of the remaining kidney to unilateral nephrectomy.

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The Stimulation of Insulin Secretion in Dogs by Tris (Hydroxymethyl) Aminomethane* (33431)

DANIEL S. ZAHARKO[1] (Introduced by Lyle V. Beck)

Department of Pharmacology, Medical Sciences Program, School of Medicine, Indiana University, Bloomington, Indiana 47401

During investigations concerning the metabolism of insulin it was noticed that when Tris[2] was used as a carrier reagent for insulin, a greater amount of insulin appeared in portal vein plasma than could be attributed to the injected insulin. This finding strongly suggested that Tris stimulated the release of endogenous insulin. According to Bennett and Tarail (1), Tris, at pH 10.2, if injected in sufficient quantity produced severe hypoglycemia in normal dogs. They suggested that the hypoglycemia might be due to insulin release and perhaps also to the facilitation of

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[1] Present address: Laboratory of Chemical Pharmacology, Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

[2] Tris (hydroxymethyl) aminomethane also known as THAM: Sigma Chemical Co., St. Louis, Missouri.