Serum Ribonuclease Activity After Temporary Ligation of Renal Pedicles in Rats.* (24165)

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The previously described increase in serum ribonuclease (*RNase*) activity after bilateral nephrectomy in several species(1) suggested investigation of the behavior of this enzymatic activity in renal failure(2) which follows temporary constriction of the renal pedicles.

It will be shown that the above procedure performed on rats leads to increases in serum *RNase* activity, reversible in surviving animals, and markedly paralleling blood urea levels.

Material and methods. Adult Wistar rats of both sexes were used. Under brief ether anesthesia both renal pedicles were ligated with thick thread. Forty-five, 60 or 90 minutes later the rats were reanesthetized and the ligatures taken off. No heparin(3) was used, as this substance inhibits serum *RNase* activity(4,5). After removal of ligatures, the kidneys returned to uniform pink color. Controls were handled similarly, but ligatures were not tied. Blood samples of approximately .3 ml were taken from tail tips after warming animals at 37°C for 10 minutes. Most animals were sampled immediately before and at end of constriction period, and at intervals up to 3 to 4 weeks after start of experiment. *RNase* activity was determined in 4 μl duplicate samples of serum as previously described(1,5), with the modification that 5% (w/v) trichloroacetic acid was added to the acetone-HCl precipitating mixture. The dialyzed substrate could be stored at −15°C for at least 6 months. Samples from each experiment were simultaneously assayed. Results are expressed in optical density units. Urea was determined in duplicate 20 μl serum samples by nesslerization after urease digestion(6).

Results. Mortalities in the 3 experimental groups, with 45, 60 and 90 min. constriction, were, 3/14 (totals of 2 experiments), 8/14 and 5/7, the one for the 60 min. group being significantly greater than Koletsky and Gustavson's data(3).

Behavior of blood urea levels. In surviving animals there was a rapid and reversible increase in serum urea, which agrees with the findings of other workers(3). In animals that did not survive, urea remained at high levels until death. The relatively small number of animals studied did not disclose relation between magnitude of urea rise and length of time the ligatures were applied. Table I shows results of one of the 45 min. experiments. The vertical lines indicate 95% confidence limits of the means. Analysis of variance of this experiment for 5 surviving† rats revealed significant ($P<.01$) effects of both "between rats" and "between times" factors. Comparisons between zero hour mean, and those of other time periods by the method of Scheffé(7), showed significantly ($P<.05$) increased urea levels up to 6 days. The 18 and 30 days group did not differ significantly from the zero hour group.

Behavior of serum RNase was similar to that of urea. No clear relationship was found between rise in enzyme activity and period of constriction on the one hand, or surviving-non-surviving condition on the other. In surviving animals enzymatic activity was reversibly increased, whereas in those which died, it remained at high levels. It should be noted that, in agreement with our previous experience, large variability was found in initial control values of different animals. Thus, a 4-fold range of values was found. When initial levels were compared with those immediately following release of ligatures, *RNase* activity was a more sensi-

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† Two dead animals and 2 surviving ones were excluded from the analysis because of missing values.

TABLE I. Serum Ribonuclease Activity and Urea Levels in 9 Rats before and after a 45 Min. Ligation of Renal Pedicles.

	Control	45 min.	12 hr	1 day	2 days	4 days	6 days	18 days	30 days
R	103	Det. lost	194	230	275	307	56	8	11
U	41	50	108	83	75	83	43	36	29
R	100	125	87	116	184	223	201	47	24
U	73	70	120	133	125	75	39	48	50
R	42	111	87	84	139	152	67	52	84
U	41	100	76	58	39	37	41	37	50
R	27	42	46	128	155	109	49	0	0
U	43	54	100	124	116	78	46	43	46
R	78	205	256	150	312*				
U	62	64	100	38	129				
R	40	134	246	218*					
U	66	66	158	Det. lost					
R	54	90	16	99	165	137	73	16	23
U	46	51	66	71	18	50	43	28	29
R	83	127	136	225	198	61	124	77	55
U	46	71	112	149	124	53	51	54	51
R	112	223	230	247	381	96	80	73	60
U	50	62	83	158	91	Det. lost	67	66	83
R	71.0 ± 10.4†	132.1 ± 20.7	144.2 ± 30.1	166.3 ± 21.2	226.1 ± 30.5	155.0 ± 27.8	92.8 ± 20.2	39.0 ± 11.7	36.2 ± 11.4
U	52.0 ± 3.9	65.2 ± 4.9	102.5 ± 9.03	101.7 ± 15.8	81.1 ± 20.6	62.5 ± 7.4	47.3 ± 3.5	44.6 ± 4.7	48.2 ± 6.7

R = Ribonuclease activity (optical density × 1000). U = Urea, mg %.
* Indicates death of animal. † Mean ± stand. error.

tive indicator than urea of the exclusion of the kidneys. After 45 minutes of total ischemia, analysis of 8 paired values by the *t* test revealed a significant increase of about 85% in the *RNase* activity, whereas a similar analysis of the urea levels showed a 27% increase, which was not significant at the 5% probability level.

Results of the 45 minute constriction experiment are given in Table I. The large confidence limits of the means are due to the above reported large disparity between initial serum enzyme activities of different animals. Analysis of variance for 5 surviving animals† showed that the effects "between rats" and "between times" were significant ($P<.01$). Study of the contrasts between means of the zero hour group and those of the other time periods(7) showed significantly increased *RNase* activity levels up to 4 days; the others were not significant.

Control animals. Fourteen control rats were studied for varying periods. In 11 of them serum *RNase* and urea were either unchanged or decreased. In the 3 remaining animals erratic variations were observed. A sample of control rats is given in Table II.

Discussion. It is known that temporary constriction of artery or renal pedicles in the rat(8) and rabbit(9) leads mainly to necrosis of the proximal convoluted tubules. Functional studies in the dog(10-13), indicate that an immediate reduction in the glomerular filtration rate follows renal ischemia, accompanied by a decrease in secretory ability of the tubules and in renal blood flow. In surviving animals these phenomena are either partially or completely reversible. It can be assumed that similar functional impairment would also be found in the rat. It should be noted that the behavior of blood urea is similar in the rat(3), rabbit(9) and the dog(11, 14).

The rapid increase in serum *RNase* activity which follows exclusion of the kidneys(1) could be explained by the assumption that plasma *RNase* is efficiently filtered by kidney glomeruli, although alternative views could also be held. On the other hand, the clearance of the enzyme being only around 2% of

TABLE II. Serum Ribonuclease and Urea Levels in Control Animals.

	Control	24 hr	36 hr	48 hr	4 days	7 days
R	73	43	53	42	27	40
U	48	37	42	23	42	42
R	88	62	52	44	114	104
U	40	29	29	33	21	25
R	82	18	34	31	92	45
U	42	37	33	25	32	50
R	244	185	190	191	166	196
U	27	30	21	36	39	37
R	122.0 ± 40.8*	77 ± 37.1	82 ± 36.1	77 ± 38.1	100 ± 28.7	96 ± 36.2
U	39.3 ± 4.5	33.3 ± 2.1	31.2 ± 4.2	30.1 ± 2.1	33.5 ± 2.3	38.5 ± 5.1

R = Ribonuclease activity (optical density × 1000). U = Urea, mg %.
* Mean ± stand. error.

endogenous creatinine clearance in the rat(15. 16), a high tubular reabsorption with inactivation should be further postulated(17.18).

That the increase in serum *RNase* activity is not secondary to the uremic condition and that the kidney is involved in inactivation of the enzyme, is shown by unpublished observations on dogs on which ureter venous anastomosis was performed bilaterally. In these experiments the increase in serum *RNase* is much smaller than that of binephrectomized controls, while degree of uremia is similar in both cases.

We wish to suggest that the above reported increase in serum *RNase* activity after renal ischemia is mainly due to reduction in glomerular filtration rate. It cannot be excluded, however, that either production, or release of enzyme to the blood, or both, may be increased by our experimental procedure, or that backflow through the damaged tubules contributes towards elevation of serum enzyme activity. Nothing is known, however, of homogeneity and physical characteristics of serum *RNase*. Pending these and other physiological data, interpretation of our findings must be speculative. The above presented hypothesis has the only merit that it may be experimentally tested.

Our findings of the parallelism between behavior of serum urea and *RNase* activity in the rat suggested that this enzyme could be profitably studied in several experimental and clinical conditions. Enzyme assays performed on plasma from normal and uremic human subjects have yielded similar results, to be reported later.

Summary. A study was performed of urea and serum ribonuclease activity after ligation of renal pedicles in rats for periods of 45, 60 or 90 minutes. Both urea and ribonuclease activity increased reversibly in surviving animals and remained elevated until death in the others. The significance of the findings is discussed, and it is suggested that the decrease in glomerular filtration rate could explain the behavior of serum ribonuclease.

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1. Rabinovitch, M., Dohi, S. R., *Am. J. Physiol.,* 1956, v187, 525.

2. Smith, H. W., *The Kidney. Structure and Function in Health and Disease.* Oxford Univ. Press, New York, 1951, p759.

3. Koletsky, S., Gustafson, G. E., *J. Clin. Invest.,* 1947, v26, 1072.

4. Metais, P., Mandel, P., *Bull. Soc. Chim. Biol.,* 1955, v37, 999.

5. Rabinovitch, M., Dohi, S. R., *Arch. Biochem. and Biophys.,* 1957, v70, 239.

6. Hawk, P. B., Oser, B. L., Summerson, W. H., *Practical Physiological Chemistry,* 13th ed., Blakiston, N. Y., 1954, p671.

7. Federer, W. T., *Experimental Design. Theory and Application,* Macmillan Co., N. Y., 1955, p40.

8. Koletsky, S., *Arch. Path.,* 1954, v58, 592.

9. Badenoch, A. W., Darmady, E. M., *J. Path. and Bact.,* 1947, v57, 79.

10. Selkurt, E. E., *Am. J. Physiol.,* 1945, v144, 395.

11. Roof, B. S., Lawson, H. D., Bella, S. T., Eder, H. A., *ibid.,* 1951, v166, 666.

12. Friedman, S. M., Johson, R. L., Friedman,

C. L., *Circ. Research,* 1954, v2, 231.

13. Moyer, J. H., Heider, C., Morris, Jr., G. C., Handley, C., *Ann. Surg.,* 1957, v45, 41.

14. Hamilton, P. B., Philips, R. A., Hiller, A., *Am. J. Physiol.,* 1948, v152, 517.

15. Rabinovitch, M., Dohi, S. R., *Abstr. of 1st Congr. of Assn. Latino-Americana de Ciencias Fisiol., Uruguay,* 1957, p148.

16. Berman, B. L., Houck, J. C., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 175.

17. Rather, L. J., *Medicina,* 1952, v31, 357.

18. Govaerts, P., Lambert, P. P., *J. d'Urol.,* 1953, v11, 693.

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Effect of Different Intrinsic Factor Preparations on Co^{60} Vit. B_{12} Uptake in Rat Liver Slices. (24166)

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Miller and Hunter(1) reported that radioactive Vit. B_{12} is absorbed by rat liver slice, and that this absorption is increased markedly by addition of intrinsic factor rich extracts of hog stomach. This work has been confirmed (2,3). That this increase in binding is related to intrinsic factor present in the concentrate is suggested by the fact that it is abolished by boiling of intrinsic factor concentrate(1), and that proteins without known intrinsic factor activity fail to produce this increased binding. These proteins include gelatin, lysozyme, crystalline pepsin and pink protein(1). Addition of human gastric juice failed to enhance Cobalt-60 labeled Vit. B_{12} uptake by rat liver slice. Since this was similar to the results obtained with human gastric juice in gastrectomized rats, it seemed advisable to extend these observations to rat gastric juice, dog gastric juice, hog intrinsic factor concentrates and hog gastric juice to test the effect these intrinsic factor preparations have on liver slice technic.

Technic. Seventy-five to 100 mg slices were prepared from chilled rat livers and incubated for 30 min. at 37° in Warburg vessels containing Hastings' buffer(4) 15 mμg radioactive Vit. B_{12} and measured amounts of gastric juice. Prior to incubation the system was flushed 5 min. with 5% CO_2 and 95% O_2 mixture. After incubation, the slices were washed in fresh buffer. We found that the number of washes or length of time used in washing does not cause a change in radioactivity absorbed. The slices were transferred to glass test tubes and assayed in scintillation well type counter. The counts were corrected for background and expressed as specific activity (counts/min./100 mg). Various amounts of rat, dog, human and hog gastric juice were incubated with the liver slices. Rat gastric juice was obtained by placing a ligature over pylorus of rat stomach. Eight hours later the gastric juice was collected. Gastric juice was collected from dogs with Heidenhain pouches which had been stimulated by raw meat. Human gastric juice was obtained from fasting patients by gastric tube after insulin stimulation. Hog gastric juice was obtained by expressing gastric juice from surface of freshly slaughtered hog stomach. The hog intrinsic factor concentrate was furnished us.* In patients with pernicious anemia, this intrinsic factor concentrate is active at a dosage of 2-3 mg. The binding ability of various gastric juice fractions as tested by the method of Gregory and Holdsworth(5), *viz.* 0.61 μg of Vit. B_{12} containing 0.01 μc of Co^{60} were added to 5 mg of gastric juice. This mixture was dialyzed against tap water for 48 hours and the contents of dialysis tubing counted in scintillation well counter. These gastric juices were tested in a patient with pernicious anemia to prove that each had intrinsic factor activity. Twenty-five mg of

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