particular types of neoplastic cells and that such endogenous components could constitute a lethal accumulation for the malignant tissue if ignited by the proper reactant.

Summary. A simple experimental procedure is reported which can demonstrate a significant difference in response between certain tumor-bearing mice and their normal controls. The procedure involves challenging the two groups of animals with a lethal dose of a compound capable of reacting with the tumor or one of its metabolites. Either the cancerous or the normal animals may survive longer, depending upon both the type of tumor and the molecular configuration of the compound utilized. The difference in survival time between the tumor-bearing and normal animals is thought to be due to a modification of the toxicity of the administered compound following reaction with a tumor component. The S91 mouse melanoma was employed as the primary model since some of the chemistry of its metabolites is known. Implications of possible utility in the study of the biochemistry of tumors and in chemotherapy are discussed.

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Renal Clearance of Ribonuclease. (23679)

LEONARD B. BERMAN AND JOHN C. HOUCK (Introduced by John C. Rose)
Department of Medicine, and Surgical Research Metabolic Laboratory, Georgetown University Hospital, Washington, D.C.

Rabinovitch and Dohi have reported an increase in the serum concentration of ribonuclease (RNAase) in bilaterally nephrectomized dogs(1). Using a new simple assay procedure for determination of RNAase in serum and urine(2), we have measured its renal clearance in normal subjects, including 5 humans and 3 dogs. In addition, observations have been made on the arteriovenous concentrations of RNAase across the intact canine kidney, as well as the changes in serum RNAase concentration following bilateral ureteral ligation.

Materials and methods. Crystalline pancreatic RNAase was obtained from Armour and Co. The activity of the enzyme was assayed upon yeast ribonucleic acid (RNA) from the California Foundation for Biochemical Research. Serum levels of RNAase were determined by diluting 0.1 ml of serum to 10 ml with a solution containing 0.02M NA_2HPO_4 and 0.14M NaCl. 0.5 ml of this solution was added to a similar volume of saline-phosphate containing 1 mg of RNA per ml. The pH remained constant at 7.8 during subsequent incubation of this mixture at 37°C for 30 minutes. After this period, 5 ml of 0.1M acetate buffer, pH 3.9, containing 3 mg/ml of bovine serum albumin (Fraction V, Armour) and 1 mg/ml of Knox gelatin was added to each mixture. The absorbence of the turbid colloid resulting from the interaction of the polymerized RNA with the acidified serum albumin was determined in a Coleman Jr. spectrophotometer at 400 mμ. The optical density so determined was translated by a standard curve into μg/ml of polymerized RNA remaining. The difference between this value and the initial 500 μg was expressed as μg of substrate depolymerized.

TABLE I. Ribonuclease Clearances in 5 Normal Humans.

Urine conc. (μg/ml)	Urine flow (ml/min.)	Venous conc. (μg/ml)	Clearance (ml/min.)
1.60	1.1	.40	4.4
.48	3.3	.40	4.0
.28	3.7	.32	3.2
.21	8.0	.32	5.2
.36	5.3	.40	4.8
.30	11.0	.44	7.5
.44	5.6	.40	6.2
.30	9.5	.40	7.1
.20	7.3	.36	4.1
.22	13.5	.32	9.2

By comparison to a standard curve, using crystallized pancreatic RNAase, the units were translated into μg of enzyme per ml of serum. Care must be taken to avoid contamination with heparin, since this polyanion behaves as a competitive inhibitor of RNAase. Ribonuclease clearances were measured in 5 healthy male humans. Each was in a fasting state but had received an oral water load prior to and during the experiment. Urine was collected without catheterization during two 60-minute periods with a sample of venous blood at the midpoint of each period. Simultaneous endogenous creatinine clearances were used as approximations of the glomerular filtration rate. All subjects remained ambulatory throughout. Ribonuclease clearances were measured in 3 female mongrel dogs, anesthetized with sodium pentobarbital. Indwelling catheters and bladder washouts were used for the collection of urine. Each animal was hydrated with 400-700 ml of normal saline intravenously at the beginning of the experiment. Following a priming dose of creatinine, the infusing fluid was changed to creatinine in normal saline. After a suitable period of equilibration, urine was collected for 3 10-minute periods with an arterial blood sample taken at the midpoint of each period. Each dog was then given various sized priming doses of RNAase intravenously and the sustaining infusions were changed to RNAase-creatinine in normal saline. One to three clearance periods were performed at each new level of enzyme. Infusion rates were regulated by gravity drip. The clearances of exogenous creatinine were used as approximations of the glomerular filtration rate. All urine flow rates shown include washout volumes. RNAase arteriovenous differences across the kidney were measured in 3 dogs, anesthetized with sodium pentobarbital. Direct punctures of a femoral artery and right and left renal veins were used to obtain samples. This procedure was repeated after the intravenous injection of large amounts of RNAase.

Four female mongrel dogs were subjected to bilateral ureteral ligation. The animals were deprived of food and water until death on the 4th or 5th day. Daily measurements were made of serum RNAase and urea nitrogen concentrations.

Results. In presentation of experimental results in Tables I and II, no correction for body surface area has been made. In the human subjects (Table I) the mean serum RNAase concentration was 0.38 μg/ml with a range of 0.32-0.44. The mean RNAase clearance was 5.6 ml/min. with a range of 3.2-9.2. These clearances represented about 4% of the simultaneously measured glomerular filtration rate and appeared independent of variations in urine flows, from 1.1-13.5 ml/min.

The animal data showed a mean serum RNAase concentration of 0.35 μg/ml (0.18-0.50). RNAase clearances, prior to loading, averaged 3.4 ml/min. (1.5-6.1) and varied from 3-11% of the glomerular filtration rate. The clearance values for any one dog did not change significantly during loading even with RNAase serum levels acutely raised as much as 12-fold. Filtration rates remained essentially constant throughout.

Femoral artery and renal vein concentrations of RNAase were essentially the same ($A/V = 1.02$) both at normal serum levels and after a 10-fold increase.

Serum RNAase concentrations were significantly elevated in 3 of the 4 anuric dogs by the second post-operative day. However, they did not continue to rise and bore no quantitative relationship to the level of blood urea nitrogen.

Discussion. Interpretation of clearance data can be made with certainty only when certain characteristics of the test substance

TABLE II. Dog Ribonuclease Clearances at Various Serum Concentrations.

Dosage	Urine conc. (μg/ml)	Urine flow & washout (ml/min.)	Arterial conc. (μg/ml)	Clearance (ml/min.)
Dog 1 (10 kg)	.5	3.8	.37	5.9
	.36	6.3	.37	6.1
	.36	5.6	.37	5.5
20 mg primer and 6 μg/min. infusion	1.12	2.7	.50	6.1
	1.60	2.0	.48	6.7
	1.60	2.1	.48	7.0
12 μg/min. infusion	.90	4.5	.64	6.3
	1.04	3.9	.62	6.6
	1.30	3.0	.64	5.1
Dog 2 (12.7 kg)	.50	2.2	.50	2.2
	.25	3.3	.50	1.7
	.23	3.2	.50	1.5
60 mg primer and 24 μg/min. infusion	6.2	3.2	6.0	3.3
	3.6	3.3	4.8	2.5
Dog 3 (29.1 kg)	.154	3.8	.18	3.2
	.178	2.4	.16	2.7
	.160	1.7	.16	1.7
60 mg primer and 12 μg/min. infusion	1.90	2.1	1.6	2.5

are known. These include the degree of *in vivo* serum protein binding, the molecular size (which for this enzyme is about 13,750), the occurrence of metabolism and/or storage within the kidney. In the absence of any precise knowledge about some of these points, explanation of the present data is necessarily tentative. The failure of the RNAase clearance to change during loading suggests that the filtered load is neither reabsorbed nor added to by the tubules. The small magnitude of the clearance relative to the filtration rate may reflect a high degree of protein binding, with consequent inability of a large portion of serum RNAase to pass the glomerular membrane. A recent paper by McGeachin and Hargan(3) proposes just such a mechanism of excretion for another enzyme, serum amylase. An alternative explanation for our data is that the filtered load of RNAase is normally at or above its tubular maximum.

The arterio-venous concentrations of RNAase across the kidney suggest that this organ neither metabolizes nor produces significant amounts of RNAase under the conditions of experiment.

Serum RNAase concentrations are elevated in anuric dogs as reported by Rabinovitch and Dohi(1) but the levels cannot be used either as an index of catabolic rate or duration of anuria.

Summary. (1) The magnitude of the renal clearance of RNAase and its failure to change during loading suggests filtration of an unbound fraction of enzyme as the mode of excretion. (2) The arterial and renal vein concentrations of RNAase across the kidney were identical under the conditions of these experiments. (3) RNAase serum levels were elevated in 3 of 4 anuric dogs but did not continue to rise with blood urea nitrogen.

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