Regional Differences in Ribonuclease Content of Rat and Mouse Kidney

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Kidney cortex, red medulla and white medulla were separated into nuclei, mitochondria, microsomal and 105000g supernatant fractions. Assay of RNAase (ribonuclease) activity at pH 7.8 revealed that, for each subcellular fraction, activity was much greater in cortex than in red or white medulla; this was true for both free RNAase and total (free plus latent) RNAase. For example, the free RNAase activity in the 105000g supernatant of cortex was 5 and 8 times higher than in red and white medulla respectively. No latent RNAase activity was found in any particulate fraction. Latent supernatant RNAase activities (suggesting presence of bound RNAase inhibitor) were similar in cortex and medulla. The cortex supernatant contained minimal free RNAase inhibitor, whereas that of the red and white medulla showed about one-third and one-tenth respectively of the inhibitor activity measured in liver. Adrenalectomy did not change RNAase activity in any fraction nor the content of free RNAase inhibitor in the kidney supernatant, but did decrease the liver RNAase inhibitor content by 40%. In supernatants from mouse kidney, both free and total RNAase activities of both cortex and red medulla were similar to those of rat red medulla. Mouse cortex contained appreciably higher amounts of free RNAase inhibitor than rat cortex. The difference between the rat and mouse cortical RNAase activity and inhibitor content may help explain the relative ease with which satisfactory renal polyribosome profiles were obtained from mouse kidneys. Our results, as well as those of Kline & Liberti [(1973) *Biochem. Biophys. Res. Commun.* **52**, 1271–1277], showing that renal red and white medulla are more active than cortex in protein synthesis, are consistent with the hypothesis that the RNAase–RNAase-inhibitor system may participate in the regulation of protein synthesis.

The precise biological roles of the alkaline RNAases* remain unclear, a situation further complicated by their wide distribution in subcellular components. The nuclear enzymes have been implicated in the processing of precursor RNA and the nuclear disposal of non-transported RNA (Liau *et al.*, 1968; Darnell, 1968; Sporn *et al.*, 1969; Burdon, 1971; Perry & Kelley, 1972; Winicov & Perry, 1974). Since RNAase inhibitor stabilizes polyribosomes during isolation (Blobel & Potter, 1966; Gribnau *et al.*, 1969), the cytoplasmic RNAase–RNAase-inhibitor system has been implicated in the regulation of protein synthesis (Roth, 1967; Brewer *et al.*, 1969; Kraft & Shortman, 1970; Liu *et al.*, 1975a,b).

Renal RNA metabolism and protein synthesis vary in response to physiological stimuli, such as hormonal administration (Avdalovic & Kochakian, 1969; Kochakian & Hama, 1969; Kochakian *et al.*, 1969, 1974; Edelman & Fanestil, 1970) and unilateral nephrectomy (Nicholls *et al.*, 1970; Rosso *et al.*, 1973). Changes in renal RNA synthesis are often considered to be part of the mechanism through which these

and other stimuli exert their effects. It is therefore important to learn more about the renal RNAase–RNAase-inhibitor system. In addition, the histological heterogeneity of the kidney must be taken into consideration. Kline & Liberti (1973) reported a marked gradient in leucine incorporation into protein as one progresses across the morphological layers of the rat kidney. Although the kidney as a whole is known to possess high RNAase activity (Roth, 1954; de Lamirande & Allard, 1959; Nicholls *et al.*, 1970), it has not yet been determined how the RNAase and RNAase-inhibitor activities in different regions of the kidney correlate with protein-synthetic capacity.

The present paper describes the distribution of RNAase and RNAase inhibitor in three histological zones of the rat kidney, the effects of adrenalectomy on the RNAase activity in rat kidney and differences between the kidney of the rat and mouse, comparing them with liver and heart RNAase activities.

Experimental

Animals

Male Fischer rats (250–300g) and male CD-1 mice (40–50g) obtained from Charles River Breeding

* Abbreviation: RNAase, ribonuclease.

Laboratories (Wilmington, MA, U.S.A.) were maintained in a room at $24 \pm 1^\circ\text{C}$ with 12 h of artificial light provided from 07:00 to 19:00 h. Wayne Chow (Allied Mills, Chicago, IL, U.S.A.) and tap water were provided *ad libitum*. Adrenalectomized rats prepared as described previously (Liu *et al.*, 1972) were used 1 week later.

Preparation of tissue

The animals were killed by decapitation. Liver, heart and kidney were excised immediately and placed in cold 0.25 M-sucrose in TKM buffer (50 mM-Tris/HCl, pH 7.6, 25 mM-KCl and 10 mM-MgCl₂). All of the following steps were performed at 4°C . Kidney was decapsulated and sliced with a Stadie-Riggs slicer. Two 0.5 mm slices from each of the dorsal and ventral sides, together with a 1 mm slice from the lateral side of the kidney, were collected as cortex and rinsed thoroughly in 0.25 M-sucrose/TKM buffer. By using fine scissors, medulla was separated into the red and white papilla regions, which were washed thoroughly. Hearts were trimmed of attached vessels and washed to remove any blood clots. Liver was cut into about 1 cm cubes and washed thoroughly until the washing was clear. The washed tissues were blotted dry, weighed, finely minced with scissors and homogenized in 4 vol. of 0.25 M-sucrose/TKM buffer in a motor-driven Potter-Elvehjem homogenizer with a Teflon pestle (clearance 0.025 cm), 15 up-and-down strokes for heart and eight for liver and kidney tissues. The homogenate was centrifuged at 600g for 10 min. The pellet was suspended in 2.3 M-sucrose/TKM and centrifuged at 40000g for 60 min; the resulting nuclear pellet was resuspended in 0.25 M-sucrose. The supernatant of the 600g centrifugation was separated into mitochondrial, microsomal and cytoplasmic fractions as described by Hogeboom (1955), except that centrifugation at 105000g was for 2 h, rather than for 1 h. The cell fractions were kept frozen overnight and assayed the next day for RNAase activity. The inhibitor in the cytoplasmic fraction was assayed with freshly prepared sample on the same day.

Materials

Yeast RNA, bovine serum albumin and *p*-chloromercuribenzenesulphonate were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), bovine pancreatic RNAase was from Boehringer Mannheim Co. (New York, NY, U.S.A.), and RNAase-free sucrose from Schwartz/Mann (Orangeburg, NY, U.S.A.). Solutions of yeast RNA and of pancreatic RNAase (Worthington, Freehold, NJ, U.S.A.) were prepared as described previously (Liu *et al.*, 1975b).

Assay of RNAase and RNAase inhibitor

The alkaline RNAase and RNAase-inhibitor activities were assayed by previously described procedures (Liu *et al.*, 1975b). For the assay of total RNAase (free plus latent), *p*-chloromercuribenzenesulphonate was used instead of *p*-chloromercuribenzoate. The free RNAase activity represents the increase in A_{260} of acid-soluble products in the assay without *p*-chloromercuribenzenesulphonate. Total RNAase activity was that obtained in the presence of *p*-chloromercuribenzenesulphonate. The difference between total and free activity was taken to be the latent RNAase activity. One unit of RNAase activity was defined as the amount that produced an increase of 1 A_{260} unit in 30 min under the assay conditions. One unit of inhibitor activity was the amount that produced 50% inhibition of 5 ng of pancreatic RNAase.

Protein was determined by the biuret method (Gornall *et al.*, 1949), with bovine serum albumin as a standard.

Protein deamination

Protein was determined by the biuret method (Gornall *et al.*, 1949), with bovine serum albumin as a standard.

Results

Free and latent RNAase of rat tissues

Table 1 compares alkaline RNAase activities in homogenates of liver and different zones of the kidney. On a tissue-weight basis, renal cortex contained 5 and 20 times as much RNAase activity as red medulla and white medulla respectively, and 10 times as much as liver. Thus the previous reports that RNAase activity in kidney exceeds that in liver (Roth, 1954; de Lamirande & Allard, 1959) are primarily due to higher cortical RNAase activity. The total RNAase activity assayed in the presence of *p*-chloromercuribenzenesulphonate was greater in each tissue than free RNAase activity, indicating the presence of RNAase-bound inhibitors. The relative increase due to latent RNAase was almost as great in the cortex and red medulla as in the liver.

The RNAase activity in separated subcellular fractions, as in the homogenates, was greatest in fractions from the cortex. The free RNAase in the nuclei from the cortex was 5 times that of red and white medulla and 3 and 8 times that of heart and liver

Table 1. Total RNAase activity in the homogenate of liver and various regions of rat kidney

The data represent the means of triplicate assays on tissue pooled from three rats.

Tissue	RNAase activity (units/g of tissue)	
	Free	Free+latent
Liver	152	777
Kidney		
Cortex	1400	2170
Red medulla	264	866
White medulla	65.8	290

nuclear fractions respectively (Table 2). Free RNAase in the cortex mitochondrial fraction was the highest among all of the cell fractions, and was 4 and 9 times that of mitochondria from the red and white medulla respectively. The great relative differences in free RNAase among the three regions of the kidney were also found in the 105000g supernatant. The cortex supernatant contained more than 5 times as much activity as those from red and white medulla and 6 and 25 times as much as those from heart and liver respectively. In agreement with previous reports (Roth, 1967), the liver supernatant contained very little free RNAase activity and the cell fractions other than the 105000g supernatant contained no detectable latent RNAase.

On addition of *p*-chloromercuribenzenesulphonate, which reveals latent RNAase activity, an increase in RNAase activity occurred in the supernatant fractions of all samples. The amount of latent activity was about the same in supernatants from all three regions of the kidney. Total activity in the supernatant was twice as much in the cortex as in red and white medulla and 3 and 4 times as much as in liver and heart supernatants respectively. These results

indicate that the kidney cortex contained highest free RNAase activity.

The effects of adrenalectomy on the free and latent RNAase activity in different fractions are shown in Tables 3 and 4. Adrenalectomy produced no significant change in RNAase activity in any of the cell fractions tested.

Comparison of free and latent RNAase in the 105000g supernatant of rat and mouse kidney

It is difficult to isolate intact polyribosomes from the rat kidney (Nicholls & Bishay, 1971; Nicholls *et al.*, 1970), but mouse kidney polyribosomes have been successfully isolated (Kochakian *et al.*, 1969; Irwin *et al.*, 1975; Quellette *et al.*, 1976). We considered it possible that the difference in polyribosome stability between the two species might be due to differences in their RNAase activities. The results of assays of the free and latent RNAase in the supernatant of both mouse and rat kidney are shown in Table 5. The cortex of the rat contained almost 3 times as much free RNAase as the mouse cortex, but the medullary RNAase in both species was about the same.

Table 2. *Distribution of RNAase activity in cell fractions from homogenates of liver, heart and different regions of rat kidney*

The data represent the mean values $\pm$ S.E.M., for the numbers of experiments in parentheses. Each experiment used tissue pooled from three rats except: kidney-cortex supernatant, nine experiments with pooled tissues and seven single-animal experiments; and red-medulla supernatant, eight experiments with pooled tissue and six single-animal experiments.

Fraction	Tissue	RNAase activity (units/mg of protein)	
		Free	Free+latent
Nuclei	Liver	1.39 $\pm$ 0.40 (8)	1.78 $\pm$ 0.54 (8)
	Heart	3.43 $\pm$ 0.75 (8)	2.75 $\pm$ 0.79 (8)
	Kidney		
	Cortex	11.42 $\pm$ 1.15 (8)	11.80 $\pm$ 1.02 (8)
	Red medulla	2.32 $\pm$ 0.62 (7)	2.58 $\pm$ 0.41 (6)
	White medulla	2.21 $\pm$ 0.51 (3)	2.08 $\pm$ 0.73 (3)
Mitochondria	Liver	6.94 $\pm$ 1.24 (8)	6.79 $\pm$ 1.08 (8)
	Heart	3.35 $\pm$ 0.65 (8)	2.09 $\pm$ 0.59 (8)
	Kidney		
	Cortex	18.35 $\pm$ 2.90 (8)	19.22 $\pm$ 2.97 (8)
	Red medulla	4.29 $\pm$ 0.74 (8)	3.64 $\pm$ 0.56 (8)
	White medulla	2.10 $\pm$ 0.40 (3)	2.46 $\pm$ 0.08 (3)
Microsomal	Liver	2.04 $\pm$ 0.78 (8)	3.98 $\pm$ 1.28 (8)
	Heart	3.84 $\pm$ 0.85 (8)	2.72 $\pm$ 0.75 (8)
	Kidney		
	Cortex	11.98 $\pm$ 1.55 (8)	12.59 $\pm$ 1.88 (8)
	Red medulla	3.04 $\pm$ 0.59 (8)	3.07 $\pm$ 0.54 (8)
	White medulla	2.44 $\pm$ 0.20 (4)	3.58 $\pm$ 0.67 (4)
105000g supernatant	Liver	0.53 $\pm$ 0.19 (9)	6.62 $\pm$ 1.13 (9)
	Heart	2.16 $\pm$ 0.62 (9)	5.47 $\pm$ 1.00 (9)
	Kidney		
	Cortex	12.64 $\pm$ 1.70 (16)	24.60 $\pm$ 1.61 (16)
	Red medulla	2.48 $\pm$ 0.52 (14)	11.26 $\pm$ 1.14 (14)
	White medulla	1.64 $\pm$ 0.81 (8)	13.06 $\pm$ 1.82 (8)

Table 3. *Effects of adrenalectomy on RNAase in different cell fractions of rat tissue in the absence of p-chloromercuribenzenesulphonate*

The data represent mean values $\pm$ S.E.M., for the numbers of experiments in parentheses; all experiments were done on tissue from single animals.

Fraction	Tissue	Free RNAase (units/mg of protein)	
		Sham-operated	Adrenalectomized
Nuclei	Liver	1.34 $\pm$ 0.38 (4)	0.86 $\pm$ 0.29 (4)
	Heart	3.93 $\pm$ 1.25 (4)	4.14 $\pm$ 1.08 (4)
	Kidney		
	Cortex	13.55 $\pm$ 1.42 (4)	13.25 $\pm$ 1.71 (4)
	Red medulla	2.68 $\pm$ 1.35 (3)	2.86 $\pm$ 0.91 (3)
Mitochondria	Liver	8.38 $\pm$ 1.94 (4)	7.15 $\pm$ 2.70 (4)
	Heart	2.84 $\pm$ 1.23 (4)	3.77 $\pm$ 0.97 (4)
	Kidney		
	Cortex	22.73 $\pm$ 4.20 (4)	22.06 $\pm$ 6.28 (4)
	Red medulla	4.97 $\pm$ 1.40 (4)	4.96 $\pm$ 1.27 (4)
Microsomal	Liver	0.58 $\pm$ 0.32 (4)	1.53 $\pm$ 0.97 (4)
	Heart	3.68 $\pm$ 1.74 (4)	3.79 $\pm$ 2.90 (4)
	Kidney		
	Cortex	12.24 $\pm$ 2.69 (4)	16.79 $\pm$ 3.84 (4)
	Red medulla	2.74 $\pm$ 1.11 (4)	3.02 $\pm$ 1.75 (4)
105000g supernatant	Liver	0.52 $\pm$ 0.26 (5)	0.48 $\pm$ 0.21 (5)
	Heart	2.25 $\pm$ 1.10 (5)	2.12 $\pm$ 1.19 (5)
	Kidney		
	Cortex	15.26 $\pm$ 1.51 (5)	15.04 $\pm$ 2.51 (5)
	Red medulla	1.23 $\pm$ 0.35 (4)	0.39 $\pm$ 0.35 (4)

Table 4. *Effects of adrenalectomy on RNAase in different cell fractions assayed in the presence of p-chloromercuribenzenesulphonate*

The data represent mean values $\pm$ S.E.M., for the numbers of experiments in parentheses; all experiments were done on tissue from single animals.

Fraction	Tissue	Free+latent RNAase (units/mg of protein)	
		Sham-operated	Adrenalectomized
Nuclei	Liver	1.90 $\pm$ 0.33 (4)	1.10 $\pm$ 0.39 (4)
	Heart	2.85 $\pm$ 1.09 (4)	3.72 $\pm$ 1.38 (4)
	Kidney		
	Cortex	13.18 $\pm$ 1.28 (4)	12.53 $\pm$ 1.83 (4)
	Red medulla	2.22 $\pm$ 0.02 (2)	2.37 $\pm$ 1.17 (3)
Mitochondria	Liver	7.61 $\pm$ 1.71 (4)	4.06 $\pm$ 1.71 (4)
	Heart	0.82 $\pm$ 0.66 (4)	0.86 $\pm$ 0.65 (4)
	Kidney		
	Cortex	23.18 $\pm$ 4.54 (4)	19.33 $\pm$ 5.51 (4)
	Red medulla	3.62 $\pm$ 1.04 (4)	2.19 $\pm$ 0.40 (4)
Microsomal	Liver	2.33 $\pm$ 0.87 (4)	1.76 $\pm$ 0.84 (4)
	Heart	2.95 $\pm$ 1.48 (4)	2.53 $\pm$ 1.39 (4)
	Kidney		
	Cortex	13.46 $\pm$ 3.32 (4)	18.98 $\pm$ 3.97 (4)
	Red medulla	3.29 $\pm$ 1.07 (4)	5.55 $\pm$ 1.84 (4)
105000g supernatant	Liver	7.19 $\pm$ 0.88 (5)	9.43 $\pm$ 2.12 (5)
	Heart	5.57 $\pm$ 1.82 (5)	10.22 $\pm$ 2.43 (5)
	Kidney		
	Cortex	25.40 $\pm$ 2.24 (5)	26.48 $\pm$ 2.70 (5)
	Red medulla	14.87 $\pm$ 3.41 (4)	12.73 $\pm$ 1.75 (4)

Table 5. Comparison of the RNAase activity in the 105 000g supernatant of the rat and mouse kidney
The data represent mean values $\pm$ S.E.M., for the numbers of experiments in parentheses; all experiments were done on tissue from single animals.

Animal	Tissue	RNAase activity (units/mg of protein)	
		Free	Free+latent
Rat	Liver	1.42 $\pm$ 0.16 (3)	12.08 $\pm$ 3.51 (3)
	Kidney		
	Cortex	13.15 $\pm$ 3.56 (7)	28.71 $\pm$ 1.69 (7)
	Red medulla	4.43 $\pm$ 0.42 (6)	12.31 $\pm$ 1.47 (6)
Mouse	Liver	1.63 $\pm$ 0.35 (3)	14.70 $\pm$ 3.01 (3)
	Kidney		
	Cortex	4.50 $\pm$ 0.92 (7)	15.46 $\pm$ 3.02 (7)
	Red medulla	5.73 $\pm$ 0.81 (7)	10.77 $\pm$ 2.11 (7)

Fig. 1. Determination of kidney cytoplasmic RNAase inhibitor activity

The 105000g supernatant fraction from different regions of rat kidney was prepared and inhibitor activity was assayed, as described in the Experimental section. Serial dilutions of the supernatant were made in 0.25M-sucrose/TKM buffer and 50μl samples of the dilutions were used in the assay. Cortex (Δ), red medulla (○) and white medulla (□).

Table 6. RNAase-inhibitor activity in the 105000g supernatant of adrenalectomized and sham-operated rat tissues

The data represent mean values $\pm$ S.E.M., for the numbers of experiments in parentheses; all experiments were done on tissue pooled from three rats. n.d., Activity was not detectable by the assay method with up to 50μl of the 105000g supernatant. According to the results of Student's *t* test, only the liver exhibits a significant difference (*P* < 0.05) in inhibitor activity between sham-operated and adrenalectomized animals. The minimum amount of inhibitor that can be detected, by extrapolation of an inhibition curve, is 10units/g of tissue.

Tissue	RNAase inhibitor activity (units/g of tissue)	
	Sham-operated	Adrenalectomized
Liver	3190 $\pm$ 204 (4)	2090 $\pm$ 212 (5)
Heart	999 $\pm$ 90 (4)	894 $\pm$ 79 (4)
Kidney		
Cortex	n.d. (5)	n.d. (5)
Red medulla	1170 $\pm$ 248 (5)	1120 $\pm$ 205 (5)
White medulla	348 $\pm$ 78 (4)	459 $\pm$ 116 (4)

RNAase-inhibitor activity in the high-speed supernatant

When the free RNAase-inhibitor activity in the 105000g supernatant of the kidney preparations was measured by its ability to inhibit pancreatic RNAase, we observed that the activity varied greatly among the three regions of the rat kidney (Fig. 1). The rat kidney cortex contained such low free inhibitor activity that we could not detect inhibition of the pancreatic RNAase. The red medulla contained about 3 times as much activity as the white medulla (Fig. 1). A summary of results, including those from the heart and liver, is shown in Table 6. The liver contained the highest amount of inhibitor, 3 and 10 times that of red medulla respectively, and 3 times that of heart. After adrenalectomy, there were no changes in inhibitor content in either

Table 7. *Comparison of RNAase-inhibitor activity of the rat and the mouse*

The data represent mean values $\pm$ S.E.M., for the numbers of single animal experiments in parentheses. n.d., Activity was not detected with up to 50 μ l of 105000g supernatant.

	RNAase-inhibitor activity (units/g of tissue)	
	Rat	Mouse
Kidney		
Cortex	n.d. (5)	717 $\pm$ 88 (4)
Red medulla	1140 $\pm$ 174 (5)	506 $\pm$ 53 (4)

the red or the white medulla, but the liver inhibitor decreased by about 40%.

RNAase-inhibitor activity in mouse kidney

Table 7 shows the results of the RNAase-inhibitor assay of the mouse kidney, compared with that of the rat. Since the high-speed supernatant from the mouse kidney cortex and red medulla was prepared and assayed simultaneously with that from the rat, we can make a direct comparison between the samples. The results show that, in contrast with the rat kidney cortex, mouse cortex contains a readily measurable amount of RNAase-inhibitor activity. However, the activity in the mouse red medulla was only about one-half that of the rat red medulla. Thus the mouse cortex not only contains less free and total RNAase activity than that of the rat kidney cortex (Table 5), but also contains much more RNAase inhibitor. These differences are consistent with the observed differential in ease of isolating polyribosomes from these two species.

Discussion

Our results agree with previous reports that the kidney, particularly its cortex, contains more alkaline RNAase than do tissues such as liver and heart (Roth, 1956; de Lamirande & Allard, 1959; Royce, 1967; Nicholls *et al.*, 1970). Our data show that the RNAase activity of the rat cortex was equally distributed among its subcellular components. Since rat cortex comprises almost two-thirds of the kidney volume (Rouiller, 1969), the high RNAase activity of the kidney homogenate arises mainly from cortical RNAase activity. Unlike the rat kidney, no marked difference in the alkaline RNAase activity occurred across the morphological zones in mouse kidney.

The RNAase inhibitor stabilizes polyribosomes (Blobel & Potter, 1966; Gribnau *et al.*, 1969) and the inhibitor/RNAase ratio is proportional to the amount of polyribosomes in the polyribosome profile; further, the distribution of inhibitor in the various

regions of the kidney has not been closely examined before. Our results show that the 105000g supernatant from the rat cortex had high total RNAase activity and very low natural RNAase-inhibitor activity. Conversely, the medulla contains a considerable amount of the inhibitor and less total RNAase than does the cortex. These results, together with the observation that polyribosome preparations from rat kidney show a high proportion of monomers and a low proportion of larger-sized polyribosomes (Zomzely *et al.*, 1968; Nicholls *et al.*, 1970; Shires *et al.*, 1974; Poels, 1976), may explain the lower protein-synthetic activity observed in cortex than in medulla (Kline & Liberti, 1973). A notable observation is that the mouse kidney, unlike the rat kidney, shows no large difference in RNAase content between cortex and the medulla. The mouse cortex also contains considerable RNAase-inhibitor activity, possibly explaining the ease of obtaining a good polyribosomal profile from mouse kidney (Kochakian *et al.*, 1969; Irwin *et al.*, 1975).

From the amount of latent RNAase activity revealed by using *p*-chloromercuribenzenesulphonate in the RNAase assay, it has been shown that the rat kidney contains some RNAase-inhibitor activity, though less than in the liver (Roth, 1954; Nicholls & Bishay, 1971). We also showed that almost as much latent RNAase activity exists in renal cortex as in the liver, indicating that the cortex indeed contains the bound inhibitor. However, using pancreatic RNAase to assay the free inhibitor, we observed no free inhibitor. Thus our results appear to contrast sharply with those of Bishay & Nicholls (1973). They were able to purify the inhibitor partially from the rat kidney cortex. A possible explanation is that they purified the bound inhibitor. The difference between our results and those of Bishay & Nicholls (1973) is not attributable to the use of different strains of rats. In most of our studies we used Fischer rats, whereas they used Sprague-Dawley rats. However, we observed similarly high RNAase and no detectable inhibitor activities in the kidneys of Sprague-Dawley rats (Liu, D. K. & Matrisian, P. E., unpublished work). Another possible explanation may be the completeness of separation of medulla from the cortex. Since we used only the very outermost cortical portion of the kidney for preparation of cortex subcellular fractions, our results for the cortex would not reflect the activities of the inner cortex or any of the medulla. Possibly RNAase and inhibitor activities differ in inner and outer cortex.

The renal tubules, particularly the proximal tubules of the rat and the dog, have been shown to have the ability to reabsorb small proteins, including RNAase, filtered through the glomeruli (Dohi *et al.*, 1959; Rabinovitch, 1959; Cortney *et al.*, 1970). Possibly the greater RNAase activity of the cortex may come from blood plasma. This hypothesis seems

more attractive in the light of our observations (Liu, D. K. & Matrisian, P. E., unpublished work) that rat serum contains, on a unit-volume basis, 3 times as much total RNAase as rat cortex. The greater RNAase activity of the cortex is distributed in every subcellular fraction. We do not know whether the greater RNAase activity in all of the cellular components is of plasma origin. We also cannot rule out the possibility of an artifact resulting from redistribution of reabsorbed plasma RNAase during tissue homogenization. Since our intent is to relate RNAase activity and the protein-synthesis mechanism and RNA metabolism in general, we have chosen a medium that is normally used in the preparation of polyribosomes and nuclei to obtain the tissue fractions. In addition, we assay the enzyme activity under conditions that are optimum only for the supernatant enzyme. This simplification, together with the fact that there is more than one species of RNAase in the tissue, makes the assessment of the true origin of cortical RNAase even more difficult. At present, more data are needed to verify whether the plasma enzyme is similar to the cortical enzymes. The difference in RNAase content between the rat and the mouse kidney may reflect merely a species difference in renal handling of protein molecules.

Finally, from the practical standpoint, the low RNAase-inhibitor activity in the supernatant and the high RNAase activity in every subcellular component of rat kidney cortex point out the possibility of erroneous interpretation of the data dealing with RNA and protein synthesis in rat kidney, unless steps are taken to block the activity of this enzyme. This caution extends to studies dealing with nuclear RNA as well, since cytoplasmic RNAase inhibitor stabilizes polyribosomes, prevents non-specific degradation of nuclear RNA and maintains transport of RNA out of the nucleus (Hymer & Kuff, 1964; Hurlbert *et al.*, 1972; Grummt, 1975).

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