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Effect of Hypertonic Sodium Chloride on Polyribosomes and Protein Synthesis of Kidneys of Rats[1] (41508)

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Abstract. The effects of a single intraperitoneal administration of hypertonic NaCl (5.3 ml of 7.55% NaCl/100 g body wt) on polyribosomes and *in vitro* protein synthesis of kidneys of rats were investigated. The results revealed that there was marked disaggregation of kidney polyribosomes of rat (fasted or nonfasted) that received a single dose of hypertonic NaCl solution 30 min prior to sacrifice in comparison to control rats that received isotonic NaCl solution. *In vitro* [^{14}C]leucine incorporation into protein was significantly decreased using either postmitochondrial supernatant or microsomes from kidneys of hypertonic NaCl-treated rats compared with control rats. Administration of a single dose of hypertonic NaCl to rats produced a significant increase in the activity of renal RNase in comparison to that of the control group. Assay of renal acid phosphatase in the particulate and soluble fractions revealed no changes in the activities in the control and experimental rats.

Earlier studies from our laboratory have reported that the administration orally or intraperitoneally of hypertonic solutions of sodium chloride, potassium chloride, magnesium chloride, sodium sulfate, or sucrose to mice rapidly induced disaggregation of polyribosomes and decreased *in vitro* and *in vivo* incorporation of [^{14}C]leucine into proteins of livers (1). In further studies, it has been demonstrated that ribosomes isolated from the livers of mice treated with a hypertonic (4%) NaCl solution were less active in initiation of polypeptide synthesis than those isolated from control animals receiving a 0.85% NaCl solution (2). Initiation factors isolated from the livers of control and experimental animals were equally active, suggesting that the ribosomes alone were affected by the hypertonicity (2). The deleterious effects of hypertonic solutions on hepatic polyribosomes and protein synthesis were shown to be mediated by a rise in the osmotic pressure of portal venous blood plasma (3).

The present investigation was designed to determine whether polyribosomes in organs other than liver will respond to changes in osmotic pressure of blood induced by administering hypertonic solutions. In this study, the effects of hypertonic NaCl injected intraperitoneally on polyribosomes and *in vitro* protein synthesis of kidneys of rats were investigated. The kidney was selected since it plays a vital role in the purification of blood and excretes excess salts derived from the blood. Furthermore, since rat serum has been reported normally to contain high levels of ribonuclease activity (4), it was decided to determine the ribonuclease activities of serum and kidney after administering hypertonic NaCl in consideration that elevations may occur which could influence the status of kidney polyribosomes and the rate of protein synthesis. The results of this communication indicate that the administration of a single dose of hypertonic NaCl to rats caused disaggregation of polyribosomes and inhibition of *in vitro* protein synthesis of kidneys along with an increased activity of renal ribonuclease.

Materials and Methods. *Animals.* Female rats of the Sprague–Dawley strain (Sprague–Dawley, Madison, Wisconsin), 11 weeks old, and weighing 170–220 g, were

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used. The rats were maintained in a temperature-controlled room with alternating 12-hr cycles of light and dark. In studies with fasted rats, the animals were fasted overnight but had free access to water. The following morning, fasted and nonfasted rats were divided into groups, each of which contained two to four animals. Rats received intraperitoneally either 0.85 or 7.55% NaCl solution (45 or 400 mg/100 g of body wt) 30 min before killing.

Chemicals. L-[^{14}C]Leucine (10 mCi/mmole) and poly-[5-^{3}H]uridylic acid (17–61 Ci/mmole) were obtained from Amersham Corporation, Arlington Heights, Illinois. Ribonuclease A, purified from bovine pancreas, was purchased from Worthington Life Sciences Division, Freehold, New Jersey.

Sucrose density gradient analysis of polyribosomes. Rats were killed by decapitation and bleeding. Kidneys were removed, dissected free of perirenal fat, and washed three times in ice-cold 0.25 *M* sucrose solution containing TKM buffer (0.05 *M* Tris–HCl, pH 7.5; 0.025 *M* KCl; and 0.01 *M* $MgCl_2$). The kidneys were weighed, minced with scissors in 4 vol of ice–cold 0.25 *M* sucrose in TKM buffer, and homogenized with a Potter–Elvehjem homogenizer with a motor-driven Teflon pestle (clearance, 0.010 in.) and four up-and-down strokes at 1325 rpm. Postmitochondrial supernatant (PMS) was prepared by centrifuging the homogenate using a SS34 rotor in a Sorvall Model SS-3 centrifuge for 10 min at 8000 rpm. A portion (0.4 ml containing 26 $A_{260\ nm}$ units) of the PMS was layered on top of a linear 12-ml sucrose gradient (0.3 to 1.1 *M* sucrose containing TKM) in a cellulose nitrate tube, centrifuged using a Spinco SW 41 rotor in a Spinco Model L3-40 ultracentrifuge at 38,000 rpm for 1 hr at 4° and the polyribosomes were analyzed using a Gilford Model 2400S UV spectrophotometer as described earlier (3). The degree of polyribosomal aggregation of kidneys in the control and experimental groups was evaluated from the sucrose density gradients by calculating the relative distribution of monomer-dimers in relation to total ribosomes.

In vitro [^{14}C]*leucine incorporation into proteins. In vitro* incorporation of [^{14}C]-leucine into proteins using PMS or microsomes of kidneys was performed by the method of Nicholls *et al.* (5). Microsomes were prepared by centrifuging PMS for 1 hr at 40,000 rpm in a Spinco Model L3-40 ultracentrifuge. Microsomal pellets were resuspended in 0.25 *M* sucrose containing TKM buffer.

PMS (0.5 mg protein) or microsomes (0.25 mg protein) were incubated with 0.5 ml of standard incubation mixture in a total volume of 1.0 ml for 1 hr at 37°. The incubation mixture for each test tube contained ATP (2 μmole), phosphoenol pyruvate (10 μmole), pyruvate kinase (40 μg protein), sucrose (125 μmole), Tris–HCl, pH 7.5 (25 μmole), K^+ (12.5 μmole), Mg^{2+} (5 μmole) and [^{14}C]leucine (0.5 μCi). Cell sap (5 mg protein) from livers of control rats was used in the reaction mixture when microsomes were incubated. The cell sap was prepared by homogenizing 1 part liver and 2.5 parts 0.25 *M* sucrose containing TKM buffer and then centrifuging at 8000 rpm for 10 min. The supernatant was then centrifuged at 40,000 rpm for 2 hr. The resultant supernatant (cell sap) was used for *in vitro* incorporation of [^{14}C]leucine into protein using kidney microsomes. At the end of the incubation period, 1 ml of 10% trichloroacetic acid containing 20 mg of celite was added to each tube and processed for the measurement of radioactivity into proteins as described earlier (3).

Determination of RNase activity. The RNase activity in PMS of kidneys of control and experimental rats was determined by the method of Liu and Matrisan (4). The 200-μl final volume of reaction mixture contained 0.1 *M* Tris–HCl, pH 7.4, 5 m*M* $CaCl_2$, 0.3 m*M* dithiothreitol, 0.1 μCi [^{3}H]poly(U) containing 180,000 cpm and 10–50 μl of PMS containing 0.5 to 2.0 μg protein. At the end of 15 min incubation at 37°, the reaction was stopped by the addition of 0.6 ml of cold 95% ethanol containing 10 m*M* Mg-acetate and 0.06 mg yeast RNA in 0.58 *M* Na-acetate. The samples were kept at −20° for 1–2 hr and then centrifuged at 10,000 rpm for 15 min. Aliquots

of the supernatants were counted for the radioactivity in the enzyme hydrolyzed, ethanol-soluble nucleotides. The RNase activity (pH 7.4, 7.6, and 9.5) in the serums of control and experimental animals was determined according to the method of Rahman (6).

Determination of acid phosphatase activity. The acid phosphatase activity in the particulate and soluble fractions of kidneys of control and experimental rats was determined by the method of Tani and Ogata (7). The 3.0-ml total volume of reaction mixture contained 200 μmole of 0.2 *M* Tris–maleate buffer, pH 6.0, 10 μmole of 0.02 *M* pyridoxine, 50 μmole of 0.1 *M* *p*-nitrophenylphosphate, and 1.2–2.4 mg protein of the soluble or particulate fraction. The reaction mixture was incubated for 30 min at 37°. The reaction was stopped by addition of 3 ml of 50% trichloroacetic acid. Aliquots of 0.1 ml were diluted to 2.0 ml with distilled water, then added 2.0 ml of saturated sodium carbonate and *p*-nitrophenol formed was determined by reading the samples at optical density of 430 nm.

Chemical determinations. Protein was determined by the method of Lowry *et al.* (8) and RNA was assayed by the method of Fleck and Munro (9).

Results. In earlier studies, isolation of polyribosomes from rat kidney, using procedures that were successful with liver, yielded only small polyribosomal aggregates (5, 10). The difficulty in obtaining intact polyribosomes from kidney has been attributed to a higher level of RNase activity in kidney than in other tissues, such as liver, brain, spleen, etc. However, Zomzely *et al.* (11) have reported difficulty in the preparation of intact polyribosomes from the brain tissue, similar to that with kidney, even though brain contains relatively low levels of RNase activity (lower than in liver). These authors overcame this difficulty during the isolation of brain polyribosomes by employing high concentrations of Mg^{2+} (10–12 m*M*) (higher than used in the preparation of liver polyribosomes) and by the omission of detergent treatment. These concentrations of Mg^{2+} maintained the integrity of the polyribosomes. Therefore, in the present study, a 10 m*M* Mg^{2+} concentration was used during the preparation and sucrose gradient analysis of polyribosomes from kidney postmitochondrial supernatants. Furthermore, treatment of kidney postmitochondrial supernatant with deoxycholate, prior to layering on sucrose gradients to examine polyribosomes as in the case with the liver, was omitted. This omission of detergent treatment of renal postmitochondrial supernatant should not influence the yield of polyribosomes since, unlike in the liver, the great majority of polyribosomes in kidney of adult rats are free polyribosomes (12).

Since rat kidney has been shown to contain a high level of RNase activity, we, in the initial experiments, examined the influence of adding one of two RNase inhibitors (liver postmicrosomal supernatant and polyvinyl sulfate) during the isolation and subsequent analysis on the status of kidney polyribosomes. This was conducted to determine whether inclusion of either of these two RNase inhibitors would result in obtaining better polyribosomal profile, preparations with heavier aggregates. The profiles of kidney polyribosomes prepared in the presence of either one of the two RNase inhibitors were similar to those prepared in their absence (unpublished data). Therefore, in subsequent studies these RNase inhibitors were not included during the preparation of kidney polyribosomes. Figure 1 illustrates the profiles of polyribosomes prepared from kidneys of control rats under our experimental conditions and reveals the large polyribosomal aggregates with very few monomers and dimers. The polyribosomal patterns of the control rats appear to be similar to those reported by others (4, 10, 12).

The effects of administering a hypertonic NaCl (7.55%) solution intraperitoneally on kidney polyribosomes of fasted or nonfasted rats are presented in Fig. 1. Sucrose density gradient patterns of kidney polyribosomes 30 min after the administration of hypertonic NaCl revealed marked disaggregation of polyribosomes (an increase in dimers and monomers in relation to total ribosomes) in comparison to those

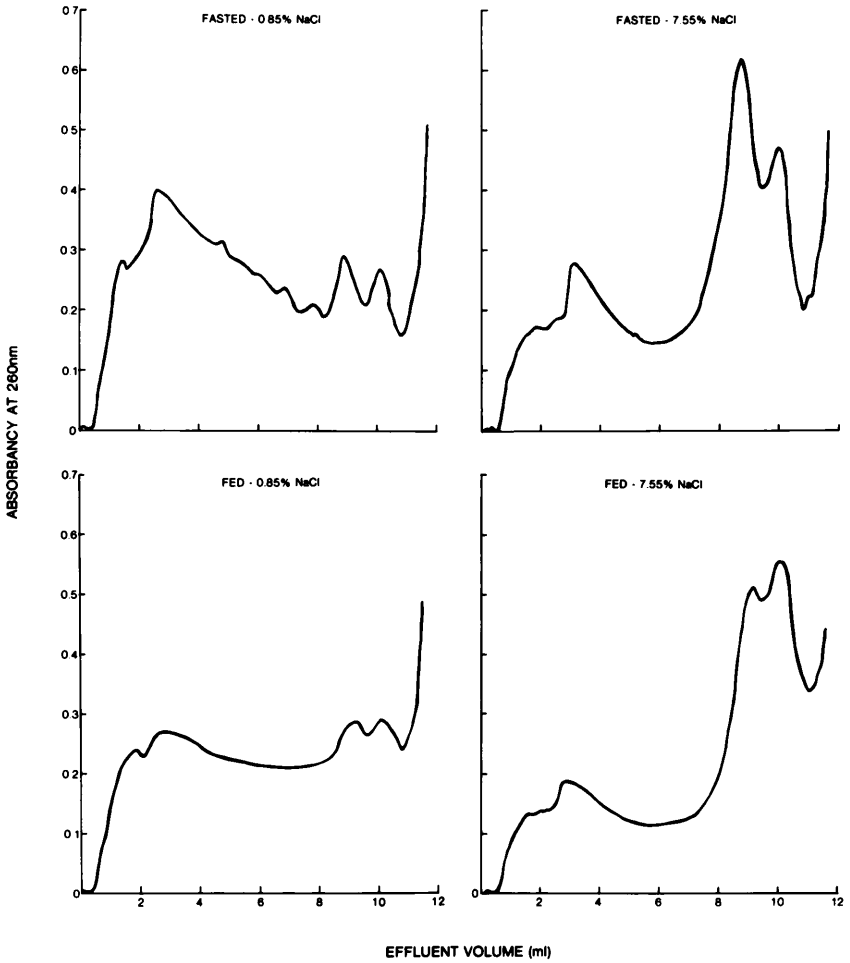

FIG. 1. Sucrose density gradient patterns of postmitochondrial supernatants of kidney polyribosomes of rats that received 0.85 or 7.55% NaCl intraperitoneally 30 min before being killed.

of control rats that received isotonic NaCl (0.85% solution). Hypertonic NaCl caused marked disaggregation of polyribosomes of kidneys of both fasted and nonfasted rats. Measurements of the areas under the polyribosomes and under the monomers plus dimers of the sucrose density gradient patterns were made in several experiments and the results are summarized in Table I. The data indicate that the administration of hypertonic NaCl to fasted and nonfasted animals resulted in a significant increase in monomer plus dimers along with a concomitant decrease in heavier polyribosomes.

In vitro [^{14}C]leucine incorporation into protein using postmitochondrial supernatants or microsomes of kidneys of control and experimental rats was also studied and the results are summarized in Table I. Both the postmitochondrial supernatants and the microsomes from kidneys of hypertonic NaCl-treated rats exhibited significant decreases in *in vitro* [^{14}C]leucine incorporation into proteins compared to values derived using preparations from control rats. Generally, the disaggregation of the renal polyribosomes correlated with the decrease in *in vitro* protein synthesizing ability in both fasted and nonfasted rats receiving hypertonic NaCl.

The administration of hypertonic NaCl

TABLE I. EFFECT OF ADMINISTERING HYPERTONIC NaCl ON WEIGHT, POLYRIBOSOMES, AND *IN VITRO* PROTEIN SYNTHESIS OF KIDNEYS

Group[a]	Weight (mg/100 g body weight)	Status of polyribosomes (Monomer − dimers/total ribosomes × 100)	[^{14}C]Leucine incorporation into protein using: Postmitochondrial supernatant (cpm/mg RNA)	Microsomes (cpm/mg RNA)
Fasted rats				
0.85% NaCl	871 ± 23 (17)[b]	31.26 ± 4.63 (5)	1479 ± 165 (11)	1878 ± 24.4 (8)
7.55% NaCl	763 ± 19* (14)	46.80 ± 2.75* (5)	457 ± 6.7* (11)	685 ± 75* (8)
% change	−12.2 ± 1.13[c,*]	+60.6 ± 20.5	−65.2 ± 5.7*	−58.1 ± 7.0*
Nonfasted rats				
0.85% NaCl	800 ± 21 (14)	32.83 ± 2.47 (6)	1614 ± 318 (6)	3971 ± 1005 (3)
7.55% NaCl	818 ± 27 (14)	52.50 ± 0.66* (6)	439 ± 122 (6)	2017 ± 420 (3)
% change	+1.72 ± 3.17[c]	+63.9 ± 11.1*	−71.5 ± 6.7*	−45.5 ± 9.1*

[a] Rats were fasted overnight or were nonfasted and then received 0.85 or 7.55% NaCl solutions (5.3 ml/100 g body weight) intraperitoneally 30 min before killing. In each experiment, kidneys of three or four rats of each group were pooled. Weights are means of the sum of both kidneys.
[b] Number of experiments in parentheses. Mean ± SEM.
[c] Mean ± SEM of differences for each experiment.
* $P < 0.05$.

did not alter the body weights of both the fasted and nonfasted rats. The mean body weights of the fasted rats were 156 ± 4.8 g (SEM) for the isotonic NaCl group and 156 ± 4.7 g for the hypertonic NaCl group, and of the nonfasted animals were 189 ± 4.7 g for the control group and 190 ± 4.6 g for the experimental group. However, the administration of hypertonic NaCl 30 min before killing caused a small, statistically significant decrease (−12%) in kidney weights in the fasted rats (Table I). On the other hand, nonfasted rats receiving hypertonic NaCl had a small insignificant increase (+2%) in kidney weights (Table I).

RNase activities of kidneys of control and hypertonic NaCl-treated rats that were fasted overnight were measured in six experiments. The results are summarized in Table II and reveal that the levels of RNase activity of the postmitochondrial supernatants were significantly higher in the kidneys of hypertonic NaCl-treated animals than the levels in the kidneys of the 0.85% NaCl-treated rats.

It has been reported earlier that rat serum normally contains a relatively high level (higher than in serum of mice) of RNase activity (4). Therefore, it was of special interest to determine whether the elevated levels of renal RNase activity in the experimental rats may be attributed to changes in the circulating serum RNase in rats treated with hypertonic NaCl. RNase activities at pH 5.4, 7.6, and 9.5 were measured in the sera of control and hypertonic NaCl-treated rats. The results of these experiments are summarized in Table III and reveal no appreciable changes in the activities of RNase in sera of the control and experimental groups.

Since the serum RNase activities were essentially unaltered after the administra-

TABLE II. RNase ACTIVITIES OF THE POSTMITOCHONDRIAL SUPERNATANTS OF KIDNEYS OF RATS THAT RECEIVED ISOTONIC OR HYPERTONIC NaCl

Group[a]	Renal RNase activities: Units/mg protein	Percentage change
0.85% NaCl	14.6 ± 3.4 (6)[b]	
7.55% NaCl	34.0 ± 7.2* (6)	+152.9 ± 42.4[c,**]

[a] Overnight fasted rats received 0.85% NaCl or 7.55% NaCl solution intraperitoneally 30 min before killing. In each experiment, kidneys of three or four rats in each group were pooled.
[b] Number of experiments in parentheses. Mean ± SEM.
[c] Mean ± SEM of differences for each experiment.
* $P < 0.05$.
** $P < 0.02$.

TABLE III. RNase ACTIVITY IN THE SERA OF RATS TREATED WITH ISOTONIC OR HYPERTONIC NaCl

Group[a]	Serum RNase activities (A_{260} units/ml)		
	pH 5.4	pH 7.6	pH 9.5
0.85% NaCl	31.97 ± 4.2 (8)[b]	36.08 ± 2.7 (13)	38.23 ± 6.4 (8)
7.55% NaCl	31.38 ± 2.2 (8)	38.35 ± 1.9 (13)	37.32 ± 6.1 (8)

[a] Overnight-fasted rats received 0.85% NaCl or 7.55% NaCl intraperitoneally 30 min before killing. Blood samples were collected by heart puncture under ether anesthesia and serum samples were separated 30 min later by centrifugation.

[b] Number of animals in parentheses. Mean ± SEM.

tion of hypertonic NaCl, we then considered the possibility that the hypertonic NaCl induced cellular injury within the kidneys by altering the osmolarity of the circulating blood. Such injury could affect the lysosomes within the renal epithelial cells and lead to an elevation in free RNase activity. To determine whether lysosomal release of enzymes may be responsible for the elevated levels of RNase activity in the kidneys of hypertonic NaCl-treated rats, we assayed for the activity of an important lysosomal enzyme, acid phosphatase, in the particulate and supernatant fractions of kidneys of control and experimental rats in three experiments. The acid phosphatase activities (A_{430} units/mg protein) of particulate and soluble fractions were, respectively, 37.0 ± 0.5 and 56.3 ± 7.7 for the isotonic NaCl group, and 44.8 ± 6.5 and 59.5 ± 12.1 for the hypertonic NaCl group. The results of these experiments indicated that the administration of hypertonic NaCl did not cause a major increase or shift in the activities of acid phosphatase in the particulate and soluble fractions compared to the corresponding fractions of the control group. This suggests that the lysosomes within the renal cells were probably unaffected by the administration of hypertonic NaCl and therefore may not be a contributory factor for the observed increase in the RNase activity of the kidneys of the experimental rats.

Discussion. The data presented in this paper demonstrate that the acute administration of a hypertonic NaCl solution caused disaggregation of polyribosomes and inhibition of protein synthesis in the kidneys. In addition, the kidneys of the experimental rats developed an increase in the activity of RNase.

Earlier studies from our laboratory have been concerned with how the liver is affected by hypertonic solutions particularly relating to the polyribosomes and protein synthesis in the livers of mice and rats. The results indicated that the oral or intraperitoneal administration of hypertonic salt solutions rapidly (within 5 min) produced an elevated osmotic pressure of the portal blood (1, 3). This increase in the portal blood osmolarity was associated with disaggregation of hepatic polyribosomes and with a decrease in both *in vitro* and *in vivo* incorporation of L-[^{14}C]leucine into hepatic and plasma proteins (1, 3). Changes in the polyribosomal profiles and in the rates of protein synthesis in response to changes in osmotic pressure caused by exposure to hypertonicity have also been observed by others in a variety of cells and organisms. Robbins *et al.* (13) and Wengler and Wengler (14) have reported that HeLa cells placed in hyperosmotic media revealed rapid disaggregation of their polyribosomes. Hsiao (15) has reported that treatment with a hyperosmotic medium caused disaggregation of polyribosomes in corn leaves. Chrispeels (16) also observed a general inhibition of protein synthesis in aleurone cells of barley by hyperosmotic media. These findings, along with our previous data with the liver and with the present findings with the kidney, suggest that protein synthesis by different cells and organs are sensitive to alterations in the osmotic pressure of the extracellular fluid.

The increased levels of RNase activity in the postmitochondrial supernatant fractions

of the kidneys of rats treated with a hypertonic NaCl solution suggests that the disaggregation of the kidney polyribosomes may be secondary due to the elevation in the levels of ribonucleases. In contrast, the polyribosomal disaggregation observed in the liver in response to the administration of 4% NaCl, reported in the earlier studies, was not found to be associated with any changes in the levels of RNase activity (2).

In an attempt to consider a mechanism by which the administered hypertonic NaCl causes polyribosomal disaggregation and decreases in *in vitro* protein synthesis in the kidney, one must consider that the elevated RNase could be of importance. Elevations of renal RNase activity in the experimental rats could be due to: (1) an increase in absorption or uptake by the kidneys of circulating RNase or (2) tissue injury causing lysosomal release of RNase. It has been reported earlier that rat serum normally contains a relatively high level (higher than in serum of mice) of RNase activity (4). Conceivably, this circulating RNase may become absorbed or extracted by the kidneys of rats treated with hypertonic NaCl. Our failure to find changes in levels of serum RNase activities in control and experimental rats in the present study does not support that the blood levels are of great importance. Yet, it is still possible that the kidneys of the experimental rats are able to extract RNase from the circulating blood without appreciably altering the levels of activities in the blood. Such a mechanism has been speculated as being responsible for the elevation of RNase in the remaining kidney after unilateral nephrectomy (17, 18) where, however, polyribosomes and protein synthesis were not depressed. Also, it is possible that the hypertonic NaCl causes increased osmolarity within the circulating blood which induces cellular injury within the kidneys. Such injury could affect the lysosomes within renal cells and cause an elevation in free RNase activity. Our failure to find an increase or shift in acid phosphatase activities of the particulate and supernatant fractions of the kidneys of control and hypertonic NaCl-treated rats does not support this possibility.

Following the administration of hypertonic NaCl, there is an elevation in NaCl concentration in the liver (1, 3) and probably also in the blood and in other organs, including the kidneys. This raises the possibility that an increase in the concentration of NaCl in the kidneys of rats treated with hypertonic NaCl may influence the assay of renal RNase activity. However, this possibility seems unlikely since the RNase activities in the sera of control and experimental rats were similar even though the NaCl concentration in the serum of the latter was elevated over that of the former group.

Further studies are needed to establish whether the disaggregation of kidney polyribosomes in hypertonic NaCl-treated rats is indeed due to elevated levels of RNase activity or to other mechanisms. Also, further studies are necessary to determine whether the disaggregation of polyribosomes and inhibition of protein synthesis of both liver and kidney by hypertonicity are mediated through similar or different mechanisms.

In the past, attempts to isolate large polyribosomes from rat kidney have been unsuccessful (5, 11). The reason for the difficulty in isolating intact polyribosomes from rat kidney compared to other tissue (e.g., rat liver or mouse kidney) is thought to be that rat kidney contains a high level of RNase activity (higher than in other tissues) (5, 19–21) and a low level of the naturally occurring RNase inhibitor (21) that stabilize polyribosomes (22). More recently Liu and Matrisian (4) reported a method to isolate RNase-free intact polyribosomes from rat kidney whereby they used an *in situ* arterial perfusion of rat kidney coupled with homogenization of the perfused rat kidney in heparin and detergents-fortified high-speed supernatant prepared from rat liver. In our hands, we were able to prepare large polyribosomes from kidneys of control rats (Fig. 1) by the employment of high concentrations of Mg^{2+} and the omission of detergent treatment during the isolation procedure. Another possible reason for obtaining heavier polyribosomes with very little degradation is that we have examined the

polyribosomal profiles using only postmitochondrial supernatants on linear sucrose gradients, thus eliminating several hours delay in the preparation of polyribosomes by the procedures normally used by others. Furthermore, our preparations of kidney postmitochondrial supernatants of control rats exhibited much lower levels of RNase activity compared to the levels in the rat kidney homogenate reported by Liu and Matrisian (4). Thus, in the present study these modifications used in our isolation procedure may have attributed to the lower levels of RNase activity in the rat kidneys and probably resulted in the recovery of heavier polyribosomes in the kidneys of control rats.

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