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SOME OBSERVATIONS ON THE "LATENT" RIBONUCLEASE OF ESCHERICHIA COLI

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The so-called "latent" ribonuclease of *Escherichia coli*[1–4] is generally considered to be truly a ribosomal enzyme, for it is found associated with only one species of particles, namely, the 30s ribosomes. Ribosomes show no "latent" RNase activity when assayed in the presence of Mg^{++}, but treatment with high salt[1] or ethylenediaminetetraacetate (EDTA), or exposure to 4 *M* urea,[2] leads to disruption of ribosomes, breakdown of ribosomal RNA, and release of the RNase. We have reported that a large fraction of the RNase passes into the sucrose-Tris medium when EDTA-lysozyme spheroplasts[5] are made, and that this occurs without any associated loss of certain internal enzymes. Malamy and Horecker[7] had previously shown the quantitative release of alkaline phosphatase on formation of EDTA-lysozyme spheroplasts. We concluded that the RNase was readily accessible to the bacterial cell surface, perhaps being associated with the protoplasmic membrane.[7–9]

It has been suggested that our results with EDTA-lysozyme spheroplasts could be explained by penetration of EDTA into spheroplasts followed by ribosomal breakdown, release of RNase, and subsequent diffusion of the enzyme through the spheroplast membrane. We had rejected this hypothesis because of the kinetics of the process. When spheroplasts were formed in sucrose-Tris medium with 10^{-3} *M* EDTA and 10 μg per ml of lysozyme, 40–50 per cent of the RNase was released in 2 min from cells in stationary phase and there was no further release during the next 60 min. By contrast, when *isolated* ribosomes were treated with 0.01 *M* EDTA, the release of enzyme was much slower and proceeded to completion. We now offer additional evidence to show that release of RNase on spheroplast formation does not result from gross ribosomal breakdown. Also, we present data that cast some doubt on the ribosomal localization of the "latent" RNase.

Experimental.—Preparation of EDTA-lysozyme spheroplasts[5, 6, 10] as well as the assays for alkaline phosphatase and β-galactosidase[5, 6] have been described.

For RNase assays the substrate of choice is sRNA because it is relatively specific, being attacked extremely slowly by phosphodiesterase and by polynucleotide

phosphorylase. For RNase assay (*A*), the incubation mixture (0.1 ml) contained 0.1 mg sRNA from *E. coli*, 0.02 *M* EDTA, and 0.1 *M* potassium phosphate buffer, pH 7.0. After 40 min at 37°, 0.35 ml of 3 per cent perchloric acid was added and the mixture centrifuged. A 0.1-ml fraction of the supernatant was mixed with 0.9 ml of water, and the $O.D._{260}$ was measured. One unit of activity is the release of 0.45 O.D. unit of acid-soluble nucleotide per 0.1 ml reaction mixture. Frequently, a prior incubation was carried out to degrade endogenous ribosomal RNA, before addition of sRNA. The two methods gave similar results. RNase assay (*B*) was similar except that EDTA and phosphate buffer were replaced by 0.005 *M* $MgCl_2$ and 0.06 *M* Tris-HCl, pH 7.1. Assay (*B*) does not detect RNase in the latent or masked form. Assay (*A*) appears to reveal all of the RNase, and this was used unless otherwise stated.

Results.—Evidence that release of RNase into spheroplast medium does not require ribosomal breakdown: When *E. coli* spheroplasts are made by suspending cells in 20 per cent sucrose-0.033 *M* Tris, pH 8, containing 10 μg per ml lysozyme and 10^{-3} *M* EDTA, there occurs a rapid release of RNase. This process ends in 3–5 min, at which time 50 per cent of the enzyme of stationary cells is found in the medium and no more passes into the medium during 1 hr of gentle shaking.[6] Almost all of the alkaline phosphatase is released at the same time, in agreement with Malamy and Horecker,[7] but β-galactosidase, polynucleotide phosphorylase, phosphodiesterase,[12, 13] glutamic dehydrogenase, and glucose-6-phosphate dehydrogenase remain entirely within the spheroplasts.

Ribosomes do not break down to a significant extent during the 3–5 min required for release of RNase into the sucrose-Tris medium surrounding spheroplasts. The yield of ribosomal RNA from intact cells and from spheroplasts was similar (Table 1). Also, ribosomes isolated from spheroplasts that had lost 50 per cent of the original RNase were identical in the ratio of RNA to protein with ribosomes prepared from normal cells. Ribosomes from normal cells and from spheroplasts gave quite similar sucrose-density patterns both in 10^{-2} *M* Mg^{++} and under conditions leading to dissociation into 50s and 30s species (10^{-4} *M* Mg^{++}).[14]

We then carried out experiments in which isolated ribosomes were exposed to conditions prevailing during spheroplast formation (10^{-3} *M* EDTA and 0.03 *M* Tris, pH 8). There was no release of RNase after 60 min at 24°C. With higher concentrations of EDTA, up to 0.02 *M*, release of RNase was observed but it was

TABLE 1

RECOVERY OF RIBOSOMAL RNASE AND RIBOSOMAL RNA FROM INTACT CELLS AND FROM SPHEROPLASTS

Expt. no.		Total RNase (units)	Total RNA (mg)
1	Ribosomes, spheroplasts	3760*	13.2
	Ribosomes, intact cells	7350*	15.2
2	Ribosomes, spheroplasts	3800	11.4
	Ribosomes, intact cells	7500	12.7

E. coli, strain C_4F_1, cells were harvested in stationary phase and divided into two equal portions: One fraction was converted into spheroplasts (Table 6); conversion was greater than 90%. Spheroplasts were collected by centrifugation and disrupted in 10^{-2} *M* Mg(acetate)$_2$-10^{-2} *M* Tris-HCl, pH 7.4, in a French pressure cell. The extract was centrifuged at 5,000 × *g* for 5 min and at 10,000 × *g* for 10 min to remove debris. Ribosomes were isolated by centrifugation for 2 hr at 100,000 × *g*. The original intact cells were treated in the same way to isolate ribosomes. The total recoveries of RNase and RNA from these ribosomes are expressed per gram wet weight of cells.

* As reported before,[4] approximately 50% of the RNase of stationary *E. coli* disappears from the ribosome fraction when spheroplasts are made, and is accounted for in the sucrose-Tris medium.

slow and required up to 6 hr for completion. It was therefore unlikely that release of 40–60 per cent of the RNase of cells in stationary phase and up to 100 per cent of the enzyme of experimental cells could be accomplished in 2–5 min merely by entry of EDTA into spheroplasts and subsequent disintegration of ribosomes. Nevertheless, it seemed of interest to examine the effect of higher concentrations of EDTA and of Tris buffer on spheroplasts.

Spheroplasts were prepared, as usual, from cells in stationary phase by treatment with 10^{-3} *M* EDTA and 10 μg per ml of lysozyme. In this case 42 per cent of the RNase was released in 2 min. In separate flasks the concentration of EDTA was increased to 0.01 *M* and to 0.04 *M*. As shown in Table 2, these higher concentrations of EDTA acting over a 20-min period did not cause a further outpouring of enzyme comparable to that obtained in 2 min with only 10^{-3} *M* EDTA. Similar results were obtained when spheroplasts were made with 5×10^{-4} *M* EDTA, which suggests that the release does not stop because an inhibitory concentration has been reached. In other experiments the concentration of Tris was increased to 0.1 *M*, again without further release of ribonuclease. These *in vitro* and *in vivo* experiments speak against rapid attack on ribosomes during the first 5 min during which spheroplasts are formed.

We then investigated the behavior of RNase that had been solubilized and fractionated through the acid ammonium sulfate step,[2] hereafter referred to as "partially purified RNase," when such material was mixed with ribosomes.

Masking of "partially purified RNase" by ribosomes: The "partially purified RNase" was active against sRNA in 5×10^{-3} *M* $MgCl_2$ and even at higher concentrations although activity was much reduced compared with assays done in EDTA-$PO_4^{\equiv}$ mixtures. Presumably this is due to aggregation of sRNA in the presence of Mg^{++}.[15, 16] In agreement with others,[1-3] we found that ribosomes were totally inactive against sRNA even in as little as 10^{-3} *M* Mg^{++}; the enzyme is in a "latent" state. A mixture of ribosomes with a 6.5-fold excess of "partially purified RNase" in 10^{-3} *M* $MgCl_2$ also was inactive against sRNA (Table 3). Thus the ribosomes were able to mask the activity of "partially purified RNase."

Binding of "partially purified RNase" by aggregating ribosomes: In another experiment a mixture of 30s and 50s ribosomes was treated with "partially purified RNase" in 5×10^{-3} *M* Mg^{++} -5×10^{-3} *M* Tris-5×10^{-3} *M* NaCl for 60 min at 0°C. The mixture was then centrifuged at 100,000 × *g* for $2^1/_2$ hr. The pellet

TABLE 2

RELEASE OF RNASE FROM SPHEROPLASTS EXPOSED TO INCREASING CONCENTRATIONS OF EDTA

	Min after Addition of Lysozyme			
	2	5	10	20
Concentrations of EDTA (*M*)	RNase activity in sucrose-Tris supernatant (units/ml)			
0.001	41	45	44	30*
0.010		34	36	26
0.040		43	31	31

Spheroplasts were prepared from *E. coli*, strain C_4F_1, cells in stationary phase by treatment with 10^{-3} *M* EDTA and 10 μg/ml of lysozyme (Table 6) which caused release of 42% of the "latent" RNase into the medium in 2 min. At this time the concentration of EDTA in separate flasks was increased to 0.01 *M* and 0.04 *M*. Samples were removed at intervals and centrifuged to remove spheroplasts; the supernatant fluids were analyzed for RNase. It is necessary to prepare spheroplasts in 10^{-3} *M* EDTA because higher concentrations prevent the optimal conversion of cells to spheroplasts.

* With continued shaking the RNase content of the medium tends to fall by 25%; this is apparently due to destruction because RNase in spheroplast lysates does not change.

TABLE 3

MASKING OF "PARTIALLY PURIFIED RNASE" IN PRESENCE OF RIBOSOMES AND Mg^{++}

Mixture	Assayed in 10^{-2} *M* EDTA (units)	Assayed in 5×10^{-3} *M* Mg^{++} (units)
0.03 ml ribosomes 0.04 ml "partially purified RNase"	360	0
Expected, if there were no interaction between ribosomes and "partially purified enzyme"	340	140

A suspension of ribosomes from *E. coli* (C_4F_1, stationary phase) assayed 1540 units per ml in 10^{-2} *M* EDTA and zero units per ml in 5×10^{-3} *M* Mg^{++}. A solution of "partially purified RNase" assayed 7,260 units per ml in 10^{-2} *M* EDTA and 3,500 units per ml in 5×10^{-3} *M* Mg^{++}.

TABLE 4

ADSORPTION OF "PARTIALLY PURIFIED RNASE" BY AGGREGATED RIBOSOMES

	Ribonuclease Activity, units			
	Initial	Supernatant, first centrifugation	Supernatant, second centrifugation	Pellet
(*A*) Ribosomes + "partially purified RNase"	740	20	0	750
(*B*) Ribosomes	230	0	0	235

A mixture (0.5 ml) was made, containing 16.7 $O.D._{260}$ units of ribosomes derived from *E. coli*, C_4F_1, cells harvested in stationary phase and "partially purified RNase" (510 units of activity), in 5×10^{-3} *M* $MgCl_2$-5×10^{-3} *M* NaCl-5×10^{-3} *M* Tris, pH 7.4. The ribosomes contained 230 units RNase. As a control, ribosomes alone were suspended in the same buffer mixture. After 1 hr at 2°C these mixtures were centrifuged in 0.5 ml cellulose nitrate tubes, in the SW39 rotor of the Model L Spinco ultracentrifuge, for 2½ hr at 36,000 rpm. The supernatant fluid was removed, and the pellets were resuspended in 0.4 ml of the same salt mixture. After being centrifuged a second time, the pellets were well drained and suspended in 0.27 ml of 5×10^{-3} *M* NaCl-5×10^{-3} *M* Tris, pH 7.4. All assays were carried out under conditions designed to reveal latent activity (assay *A*).

was resuspended in the same medium, sedimented a second time, and then suspended in 0.005 *M* Tris, pH 7.4, −0.005 *M* NaCl. The supernatant fluids contained little or no RNase activity; essentially all of it was recovered in the ribosomal pellet (Table 4). The original ribosomes, unwashed, contained considerable amounts of β-galactosidase and phosphodiesterase activity,[17] of which over 80 per cent was removed by this one wash. Thus, the adsorbed "partially purified RNase" completely resists a washing procedure that removes other enzymes from ribosomes.

Localization of "partially purified RNase" adsorbed to ribosomes that had been aggregated and then dissociated: The ribosomal pellet (previous paragraph), containing an excess of "soluble RNase" adsorbed to it was layered on top of a 5–20 per cent density gradient of sucrose containing 10^{-4} *M* Mg^{++} and 10^{-2} *M* Tris-HCl, pH 7.4 and centrifuged in the SW39 swinging bucket rotor of the Spinco ultracentrifuge Model L at 4°C for 2½ hr at 34,000 rpm. As a control, washed ribosomes that were not treated with "soluble RNase" were centrifuged in a similar gradient at the same time. The experiment revealed the usual pattern for ribosomes in low magnesium, showing a 50s and 30s peak (Fig. 1). In agreement with others[1-4] we found that the ribosomal RNase was limited to the 30s ribosomes (bottom curve, Fig. 1). However, when an excess of "soluble RNase" was adsorbed to normal ribosomes, this activity was also limited to the 30s particles (middle curve, Fig. 1). The experiment has been repeated a number of times with ribosomes from cells in exponential and stationary phase, with a variable excess of "soluble RNase," and with unwashed and 4 × washed ribosomes.

Adsorption to aggregated ribosomes: Ribosomes in 100s, 70s form in the presence of 10^{-2} *M* Mg^{++} were mixed with soluble RNase at 0°C for 1 hr. The mixture was then subjected to density gradient centrifugation in sucrose containing 10^{-2}

M Mg^{++}. All of the added "partially purified RNase" was found in the ribosomal peak showing that it had adsorbed to the aggregated ribosomes. Thus, it was not necessary for the 30s particles to be in a free state for binding of the enzyme to occur.

Binding studies under conditions that lead to ribosomal dissociation: We then carried out binding studies under conditions where ribosomes were dissociated into 50s and 30s particles. The source of "soluble RNase" for this particular experiment was the sucrose-Tris medium surrounding spheroplasts. The RNase activity was not sedimentable after 16 hr of centrifugation at 100,000 × g. Normal cell ribosomes in 10^{-4} M Mg^{++}, which were in the form of 50s and 30s particles, were mixed with a 2.5-fold excess of "soluble RNase" and suspended in 5 × 10^{-3} M NaCl-5 × 10^{-3} M Tris, pH 7.4. In the same way, a suspension of spheroplast ribosomes of the same RNA and protein content but containing only half as much RNase was mixed with a 5-fold excess of "soluble RNase." The mixtures were centrifuged for 16 hr at 100,000 × g. The supernatant fluids contained no RNase activity; all of it could be accounted for in the pellets (Table 5). Thus, "soluble RNase" can be adsorbed on dissociated ribosomal particles, and ribosomes from spheroplasts do not differ from normal cell ribosomes in this regard. Subsequent sucrose density gradient centrifugation of the resuspended pellets revealed that all of the bound RNase was associated with the 30s ribosomes.

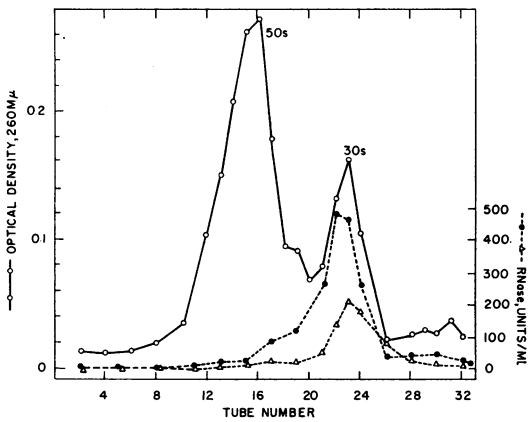

FIG. 1.—The sedimentation pattern of RNase activity when a mixture of ribosomes and "soluble enzyme" was subjected to sucrose density gradient centrifugation. *E. coli* cells, strain C_4F_1, were harvested in stationary phase, washed, and disrupted in a French pressure cell. The ribosomes were sedimented and suspended in 5 × 10^{-3} M Tris, pH 7.4, 5 × 10^{-3} M NaCl ($O.D._{260}$ = 100). A portion (0.08 ml) of the suspension was diluted to 0.5 ml in 5 × 10^{-3} M Tris, pH 7.4, 5 × 10^{-3} M NaCl-5 × 10^{-3} M $MgCl_2$. Another, similar portion was treated in the same way except that 825 units of "soluble enzyme" were also added. The mixtures were sedimented in the SW39 rotor at 35,000 rpm for $2^1/_2$ hr. The pellets were suspended in 0.25 ml of 5 × 10^{-3} M Tris, pH 7.4–5 × 10^{-3} M NaCl. These suspensions accounted for all of the original activity (Table 4), and a 0.15-ml fraction was used for gradient studies. Sucrose gradients were 5–20% sucrose (w/w) in 10^{-2} M Tris, pH 7.4–10^{-4} M Mg $(acetate)_2$, loaded with a 0.15-ml sample on a 4.6-ml gradient, and run at 34,000 rev/min in the SW39 rotor, at the 4°F setting of the Spinco Model L. Eight drop samples were collected: 0.06 ml diluted with 0.8 ml H_2O was used to read optical density at 260 mμ, and 0.005–0.015-ml samples were tested for RNase. *Upper curve:* $O.D._{260}$ for mixture of ribosomes plus "soluble RNase." Curve for "ribosomes alone" (not shown) was similar. *Middle curve:* RNase activity for ribosomes plus "absorbed 'soluble RNase'." *Bottom curve:* RNase activity for "ribosomes alone."

Location of RNase in extracts of cells disrupted in 10^{-4} M Mg^{++}: *Escherichia coli* in stationary phase were ruptured in 20 per cent sucrose-10^{-4} M Mg^{++}-10^{-2} M Tris, pH 7.4 by means of prolonged sonication or by use of the French pressure cell. The extracts were centrifuged at 100,000 × g for 16 hr to sediment the ribosomes, which were dissociated in the form of 30s and 50s particles. Ribonuclease was measured by assay A (presence of EDTA). All of the RNase was associated with

TABLE 5

ADSORPTION OF SOLUBLE RNASE TO UNAGGREGATED RIBOSOMES

Source of ribosomes	Spheroplast ribosomes		Intact cell ribosomes		Intact cell ribosomes, 4 × washed	
	RNase units added to centrifuge tube					
Ribosomal units	61	61	140	140	120	120
Units added as soluble enzyme	—	360	—	360	—	420
	RNase units recovered after centrifugation					
Units in ribosomal pellet	58	332	148	392	112	412
Units in supernatant	0	7	0	15	0	108

Ribosomes were suspended in 5×10^{-3} *M* NaCl, 5×10^{-3} *M* Tris, pH 7.4. Mg^{++} content was 2×10^{-4} *M*. Sucrose density gradients had shown that the ribosomes existed only as 30s and 50s particles. The ribosomes, 0.05 ml, were mixed with 0.3 ml of soluble enzyme and diluted to 2.0 ml in 5×10^{-3} *M* Tris, pH 7.4, 5×10^{-3} *M* NaCl. After 6 hr incubation at 0°C the mixtures were centrifuged for 16 hr at 100,000 × *g*. The supernatants were removed and the pellets resuspended in the same buffer. Assays were performed in the usual manner.

the pellet, and no significant amount was found in the supernatant fluid. This experiment suggests two possibilities: (1) all of the "latent" RNase occurs in ribosomal particles *in vivo*, or (2) all of it becomes adsorbed when extracts are made. This will be a very difficult problem to resolve.

The data thus far presented show that the latent RNase is rapidly released on spheroplast formation and may not, in fact, exist bound to ribosomes. However, neither RNase nor alkaline phosphatase comes free simply by causing injury to the cell wall or preventing cell wall synthesis by means of penicillin.

Experiments on spheroplasts made without the use of EDTA: Malamy and Horecker have reported that, in contrast to the situation with EDTA-lysozyme spheroplasts, there is no release of alkaline phosphatase when penicillin spheroplasts are made. Table 6 shows that treatment of *E. coli* with Dowex-50 and lysozyme (following Repaske[18]) yields spheroplasts that are sensitive to osmotic lysis, but again there is no release of alkaline phosphatase. Further, there is no release of RNase or DNase, as occurs with EDTA-lysozyme spheroplasts.

Effect of treating E. coli with EDTA alone, in sucrose-Tris medium: It is possible to treat stationary phase *E. coli* constitutive for alkaline phosphatase with 2×10^{-3} *M* EDTA, followed by water, and thus effect the removal of about half of the alkaline phosphatase without significant loss of RNase, DNase, or β-galactosidase. The organisms were suspended in 20 per cent sucrose-0.033 *M* Tris-HCl, pH 8.0 and exposed to 2×10^{-3} *M* EDTA for 10 min at 24°C. The cells were then sedimented by centrifugation. The pellet was resuspended in H_2O either at 24 or 4°C for 10 min and recentrifuged. Forty-five per cent of the alkaline phosphatase was in this H_2O "wash." No appreciable RNase, DNase, or β-galactosidase were evident.

The mechanism of this release is currently under investigation with use of other metal binding agents as well as other strains of *E. coli*.

Discussion.—These experiments make it uncertain whether the "latent RNase" is actually an intrinsic part of the 30s ribosome. It is possible that at least part of the RNase is located at the protoplasmic membrane, but because of the tremendous binding capacity of 30s ribosomes for this enzyme, even in the near absence of Mg^{++}, it is adsorbed to ribosomes during the preparation of cell extracts. A similar situation exists in the case of pancreatic ribonuclease for, as pointed out by Siekevitz,[19] it is unclear if this enzyme is bound to ribosomes *in vivo* or adsorbed to them on disintegration of the gland. Attachment, *in vitro*, of DNase to 70s and

TABLE 6

DOWEX-LYSOZYME SPHEROPLASTS: RELEASE OF ENZYMES (Units/ml)

Experimental procedure	β-Galactosidase		Alkaline phosphatase		RNase		DNase	
	Sucrose-Tris supernatant	Sonicate or lysate	Sucrose-Tris supernatant	Sonicate or lysate	Sucrose-Tris supernatant	Sonicate or lysate	Sucrose-Tris supernatant	Sonicate or lysate
(*A*) Dowex-lysozyme. Sonic extract, spheroplasts	0.2	20.0	1.6	38.7	0	14.9	6	175
(*A*) Dowex-lysozyme. H_2O lysate, spheroplasts	0.2	24.6	1.4	35.0	0	13.8	3	239
(*B*) EDTA-lysozyme. H_2O lysate, spheroplasts	0.2	17.2	33.2	4.4	15.3	4.6	206	—
(*C*) Sucrose-Tris control. Sonic extract, pellet	0.2	10.3	0.6	15.1	0	7.5	8	158

E. coli, strain C_4F_1, were grown in a low phosphate, 0.6% glycerol medium with isopropylthiogalactoside as inducer for β-galactosidase. Cells were harvested in the stationary phase and washed twice with 0.01 *M* Tris, pH 8.0. Cells were suspended in 20% sucrose, 0.033 *M* Tris, pH 8, 1 gm wet weight to 80 ml, and aliquots removed for various treatments: (*A*) Cells suspended in sucrose-Tris medium were exposed to Dowex-(H)-50 for 1 min and then treated with 100 μg per ml lysozyme.[9] Lysis was measured by change of O.D.$_{600}$ on dilution of the cells in water. Samples were plated for per cent survival. (*B*) Another part of the suspension was treated with 10^{-3} *M* EDTA and 10 μg per ml of lysozyme.[4] After 10 min of gentle agitation the spheroplasts were collected by centrifugation and lysed with water. Dowex-lysozyme spheroplasts were ruptured either by osmotic lysis or brief sonic treatment. (*C*) As a control, part of the sucrose-Tris suspension of *E. coli* was centrifuged directly. A sonic extract was made of the pellet. All volumes are equal and represent equal cell concentrations. Assays are as previously described.[4]

100s ribosomes has also been described, but when the ribosomes are dissociated into smaller particles this enzyme comes free.[20] We have been able, on the other hand, to show that RNase in a soluble form can be bound to ribosomes, whether already aggregated, during aggregation, or even when aggregation has been prevented. We are investigating whether other small basic proteins are adsorbed in a similar fashion.

Three enzymes, alkaline phosphatase, the latent RNase, and a DNase[20] are rapidly released when EDTA-lysozyme spheroplasts are made from *E. coli*. However, it appears that these enzymes do not come free simply by conversion of the cells to a water-lysable form. Thus, Malamy and Horecker[7] found that formation of penicillin spheroplasts is not accompanied by release of alkaline phosphatase. We now observe that treatment of *E. coli* with Dowex-50 and lysozyme[11] also leads to osmotic fragility without release of alkaline phophatase, RNase, or DNase.[21] Perhaps these enzymes are bound in some way, and a substance such as EDTA dissociates the bond. In this connection it is of interest that nearly half of the alkaline phosphatase (but no appreciable amount of RNase or DNase) was released when cells were suspended in 20 per cent sucrose-0.002 *M* EDTA and then washed with distilled water.

Our data weaken the current evidence for ribosomal localization of the "latent" RNase but certainly do not exclude the ribosomal site. If the enzyme plays a role in the turnover of mRNA or sRNA, it is of interest that degradation of ribosomes is inhibited by RNA outside the ribosomes.

These results show that gross destruction of ribonucleoprotein particles has not taken place, but it would be desirable to apply more sensitive criteria of injury. Experiments are now under way with Drs. S. Pestka and M. Nirenberg to examine the competency of spheroplast ribosomes in amino acid incorporation. Further-

more, the 30s ribosomes from spheroplasts resemble those from intact cells in being able to adsorb RNase (Table 5).

Summary.—Most of the so-called "latent" ribonuclease of *E. coli* can be released from spheroplasts in 5 min although the spheroplast ribosomes remain intact as demonstrated by RNA content, sucrose-density gradient profiles, and ability to adsorb soluble RNase. Ribosomes are able to adsorb up to a 12-fold excess of soluble RNase even under dissociated conditions. All of the RNase binds to 30s particles. Spheroplasts prepared by lysozyme alone release neither alkaline phosphatase nor RNase, but nearly half of the alkaline phosphatase can be removed by washing EDTA-treated *E. coli*, although other enzymes are not released.

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ROLE OF FERREDOXIN IN PHOTOSYNTHETIC PRODUCTION OF OXYGEN AND PHOSPHORYLATION BY CHLOROPLASTS

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It was previously reported from this laboratory[1, 2] that: (1) ferredoxin, the red, iron-containing protein native to chloroplasts, has a redox potential about 100 mV more electronegative than pyridine nucleotide and is, therefore, the strongest,