Polyribosomes and Protein Synthesis in the Spleen

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SUMMARY

Protein synthesis in rat spleens has been found to occur on polyribosomes. These structures, aggregates of two, three, four, or more ribosomes, become labeled after 1 min of incubation with radioactive amino acids. Incorporation occurs into nascent polypeptide chains which can be released by puromycin or "chased off" by 14C-amino acids. These polyribosomes stimulate cell-free protein synthesis in vitro in the presence of spleen or liver "pH 5 fraction," an energy-generating system, and an optimal magnesium concentration. Synthesis is inhibited by puromycin and by ribonuclease, which dissociates polyribosomes into monomers. Rat spleen polyribosomes thus have properties similar to those of ribosomal aggregates studied in other biological systems.

In recent years considerable evidence has appeared indicating that ribosomal aggregates, or polyribosomes, are the active units of protein synthesis in bacteria (1-3), plants (4), and animals (5-10). These structures are composed of ribosomes, messenger ribonucleic acid, and polypeptide chains undergoing active synthesis. The protein synthetic process requires the participation of soluble RNA. Soluble RNA transfers the amino acid specified by the messenger to the growing polypeptide chain which is bound, by means of a soluble RNA molecule, to the ribosome. Several ribosomes remain attached to a single messenger RNA molecule, thus giving rise to polyribosomes.

The spleen is capable of producing γ-globulin and antibody. As a prerequisite to an investigation of antibody synthesis in the spleen, it was important to study the general mechanism of protein synthesis in this organ. The spleen was found to contain polyribosomes which, like ribosomal aggregates in other mammalian organs, are active in protein synthesis when studied in vivo, in tissue fragments, and under cell-free conditions.

EXPERIMENTAL PROCEDURE

Materials—Male Sprague-Dawley rats (weighing 100 to 200 g), white New Zealand rabbits, and adult random bred Swiss (NIH) mice were obtained from the NIH animal production unit. Hanks' solution was supplied by the NIH media production unit. For some experiments, glucose, vitamins, and amino acids (11, 12) were added to the Hanks' solution (Medium 320).

Crystalline phosphoenolpyruvate kinase, sodium phosphoenolpyruvate, ATP, and GTP were purchased from Sigma. Crystalline bovine pancreatic ribonuclease was obtained from Worthington, sodium deoxycholate from Fisher, hydroxide of Hyamine from Packard, and bovine serum albumin from Armour. Other chemicals were reagent grade.

14C-Acid protein hydrolysate (specific activity, 1 mC per mg) and individual l-amino acids uniformly labeled with 14C (activity, 100 to 200 mC per mmole) were obtained from New England Nuclear.

Preparation of Polyribosomes—Rats were decapitated, and their spleens were removed. The spleens were immediately immersed in chilled Medium A (0.25 M sucrose, 0.025 M KCl, and 0.005 M MgCl2 buffered at pH 7.8 with 0.05 M Tris). All subsequent operations were performed at 4°.

The spleens were trimmed, weighed (average weight, 0.6 ± 0.2 g), and homogenized in 4 volumes of Medium A with 10 strokes of a loosely fitting Dounce homogenizer. The resulting homogenate was filtered through two layers of cheesecloth and centrifuged at 2,000 rpm (600 × g) for 5 min. The supernatant was then centrifuged at 15,000 rpm (27,000 × g) for 15 min to sediment mitochondria. The resulting supernatant was treated with 1% sodium deoxycholate for 20 min. In later experiments deoxycholate was omitted with only slight change in the yield of polyribosomes.

Of this supernatant, 3 ml were then pipetted onto 3.1 ml of 0.5 M sucrose and 2.3 ml of 2.0 M sucrose (9) buffered at pH 7.8 with 0.05 M Tris and containing 0.025 M KCl and 0.005 M MgCl2. The tubes were centrifuged in the 40 rotor of a Spinco model L ultracentrifuge at 40,000 rpm for 3 hours. In some experiments centrifugation was carried out for 8 or 17 hours. Polyribosomes were obtained as compact pellets at the bottom of the tubes. They were resuspended by gentle homogenization in a modified Medium A (sucrose concentration 0.12 M). The A260/A280 ratio was 1.8 to 1.9, and the yield was approximately 0.8 mg per g of spleen.

Radioactive Labeling of Nascent Polypeptide Chains—These experiments were performed in intact animals and in tissue fragments. In the former, rats were injected intraperitoneally with 10 to 20 μC of 14C-acid protein hydrolysate and sacrificed after 10 to 20 min. In the latter, spleens were cut into small fragments (approximately 2-mm cubes), washed, and suspended in 2.5 ml of Hanks' balanced salt solution containing 4 to 8 μC of 14C-amino acids. Medium 320 (containing 18 14C-amino acids) was used when labeling was carried out with 14C-L-lysine and 14C-L-isoleucine. The spleen fragments were incubated at 37° with shaking for 1 to 10 min, and protein synthesis was stopped.
Fig. 1. Sucrose density gradient analysis of rat spleen polyribosomes (solid line). The bottom of the gradient is on the left. Polyribosomes containing 1 mg of RNA were applied to the gradient. An aliquot was treated with 5 μg of ribonuclease at 37° for 5 min and centrifuged at the same time (broken line). The conditions of centrifugation are given in "Experimental Procedure."

Fig. 2. Sucrose density gradient analysis of rabbit spleen polyribosomes. The conditions are the same as those in Fig. 1.

**TABLE I**

Specific activity of rat spleen polyribosomes relative to time of incubation

Approximately 2 g (wet weight) of rat spleen fragments were incubated with 14C-amino acids for the times indicated, and polyribosomes were prepared. The total 14C incorporation was determined after sucrose density gradient analysis by summation of the radioactivity in each fraction. Specific activities are expressed as counts per min per mg of polyribosomal RNA.

<table>
<thead>
<tr>
<th>14C-Amino acid</th>
<th>Time (min)</th>
<th>14C incorporation (cpm)</th>
<th>Specific activity (cpm/mg RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid protein hydrolysate</td>
<td>1</td>
<td>941</td>
<td>473</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1290</td>
<td>687</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4769</td>
<td>2580</td>
</tr>
<tr>
<td>Lysine and isoleucine</td>
<td>3</td>
<td>134</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>546</td>
<td>546</td>
</tr>
</tbody>
</table>

by rapid chilling in an ice bath. The fragments were washed once in Medium A prior to homogenization.

**Sucrose Density Gradient Analysis of Polyribosomes**—The procedure used was based on that of Britten and Roberts (13). A 10 to 34% linear sucrose density gradient was prepared by mixing 13.5 ml of each sucrose solution (containing 0.025 μ
PROTEIN SYNTHESIS BY RAT SPLEEN POLYRIBOSOMES

Fig. 3. Distribution of radioactivity in fractions obtained after sucrose density gradient ultracentrifugation of rat spleen polyribosomes. The latter were prepared from tissue slices incubated in Hanks' medium at 37°C for 1 and 5 min, respectively, with ¹⁴C-acid protein hydrolysate. The position of single ribosomes (centrifuged simultaneously in a third gradient tube) is indicated by the arrow.

KCl and 0.005 M MgCl₂ and buffered at pH 7.8 with 0.05 M Tris). Polyribosomes (1 ml, containing approximately 1 mg of RNA) were carefully pipetted onto the gradient. The tubes were placed in an SW 25.1 rotor and centrifuged in a Spinco model L ultracentrifuge at 25,000 rpm for 2 hours. The gradients were collected by puncturing the tubes at the bottom and allowing the effluent to pass through a cell inserted into a Gilford model 2000 automatic spectrophotometer recording at 260 nm. Flow rate was controlled at 2 ml per min with a Sigmamotor pump. One-milliliter fractions were collected.

For measurement of radioactivity, 0.25 mg of bovine serum albumin was added to each tube as carrier, and proteins were precipitated with 5% trichloroacetic acid overnight in the cold. The precipitates were collected on 0.45-µ Millipore filters and washed twice with 5% trichloroacetic acid. The filters were dried and pasted on planchets, and radioactivity was determined in a low background Nuclear-Chicago gas flow counter (background was less than 2 cpm).

Preparation of "pH 5 Fraction"—The method of Keller and Zamernik (14) was followed. All procedures were performed at 4°C. Two volumes of a solution containing 0.9 M sucrose, 0.07 M KCl, 0.004 M MgCl₂, and 0.006 M mercaptoethanol were added to rat spleen or liver postmitochondrial supernatant fraction. Microsomes were sedimented by centrifugation at 40,000 rpm for 2 hours. The supernatant was adjusted to pH 5 with 1 M acetic acid, allowed to stand at 4°C for 15 min, and then centrifuged at 10,000 rpm (12,000 x g) for 10 min. The precipitate was rinsed and suspended in a solution containing 0.35 M sucrose, 0.07 M KCl, 0.004 M MgCl₂, 0.006 M mercaptoethanol, and 0.05 M Tris (pH 7.8). The volume was such as to give a final protein concentration of 15 to 20 mg per ml.

Cell-free Amino Acid Incorporation—The system employed was essentially that described by Maxwell (15). Incubation mixtures contained 0.35 M sucrose, 0.05 M Tris buffer (pH 7.8), 0.07 M KCl, 0.007 M MgCl₂, 0.006 M mercaptoethanol, 0.001 M ATP, 0.0001 M GTP, 0.005 M P-enolpyruvate, 20 µg of crystalline phosphoenolpyruvate kinase, 0.00008 M ¹⁴C-amino acid, 0.4 mg of polyribosomal protein, and 1.2 mg of pH 5 fraction protein.
Fig. 4. Distribution of radioactivity in fractions obtained after sucrose density gradient ultracentrifugation of rat spleen polynribosomes. In this experiment eight rats were injected with 20 μC of 14C-acid protein hydrolysate 20 min before sacrifice. Polynribosomes containing 2 mg of RNA were applied to each gradient. However, one sample was first treated with 10^{-3} M puromycin for 5 min in a complete amino acid-incorporating system (see “Experimental Procedure”). These polynribosomes were centrifuged at 40,000 rpm for 1 hour to remove released protein before being placed on the gradient.

The final volume was 0.5 ml, and incubation was carried out at 37°C. Protein was precipitated by the addition of 5 ml of 5% trichloracetic acid. The samples were kept at 4°C for 30 min or longer, heated to 90°C for 30 min, washed twice with cold 5% trichloracetic acid and once with ethanol, and dissolved in 1 ml of hydroxide of Hyamine. Then 10 ml of 0.6% 2,5-diphenyloxazole in toluene were added, and radioactivity was measured in a Packard Tri-Carb liquid scintillation counter. All assays were performed in duplicate and agreed to within 10%. The counting efficiency was approximately 50%.

Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (16) and RNA by measurement of the optical density at 260 mμ (20 absorbance units were arbitrarily taken to correspond to 1 mg of RNA (9)).

RESULTS

Distribution of Polynribosomes—A typical sucrose density gradient analysis of rat spleen polynribosomes is shown in Fig. 1. Starting from the bottom of the gradient, one observed a prominent ascending shoulder terminating in a small peak, two major peaks, and a small descending shoulder. These correspond, respectively, to aggregates of greater than four ribosomes, trimers, dimers, and monomers, as judged from their relative positions in the gradient and the known position of monomers. The latter was determined by treatment of the polynribosomes with ribonuclease. This resulted in a decrease of optical density in the heavier regions of the gradient and the appearance of a new peak (Fig. 1). This peak was dialysed overnight against Medium A without sucrose and was studied at an RNA concentration of 0.05 mg per ml by sedimentation velocity analysis in the Spinco model E ultracentrifuge.

It was found to have an s_{20,w} value of 79, similar to that reported for other mammalian single ribosomes (9, 17, 18).

Several variations in the distribution of ribosomal aggregates were observed. The major peak might be dimer, trimer, or tetramer. Two of these peaks at times were fused into a single broader peak, or a shoulder appeared instead of a separate peak. In some experiments a single broad hump was found with the maximum amplitude in the tetramer region. A prominent monomer peak was rarely found with the rat preparation.

In contrast to the rat, polynribosome preparations from mouse and rabbit spleens often showed a major monomer peak, with the amount of polynribosomes varying inversely with aggregate size (Fig. 2). A prominent monomer peak has been observed previously in preparations from rabbit spleen (19). This pat-

1 This study was kindly performed by Dr. Henry Metzger.
term, unlike that observed in the rat, is suggestive of polyribosome breakdown caused by ribonuclease. However, attempts to inhibit ribonuclease by homogenization in the presence of 60 and 240 mg of bentonite (20) did not alter the pattern obtained. Similarly, varying the magnesium concentration failed to influence the distribution of polyribosomes.

**TABLE II**

**Release of nascent protein from polyribosomes after "chase" with 3H-amino acids**

Approximately 2.4 g (wet weight) of rat spleen fragments were incubated with 14C-lysine and 14C-isoleucine for the periods indicated. After 3 min, 8 μmoles of each 14C-amino acid (a 200-fold excess) were added to Flask B, and an equal volume of water (0.4 ml) was added to Flask C. Incubation of Flasks B and C was then continued for another 7 min. Polyribosomes were prepared and analyzed by sucrose density gradient ultracentrifugation. Total 14C incorporation was determined as indicated in Table I.

<table>
<thead>
<tr>
<th>Flask</th>
<th>Time</th>
<th>Additions</th>
<th>Specific activity</th>
<th>Percentage of Flask A</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>None</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>14C-amino acids</td>
<td>81</td>
<td>60</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>H2O</td>
<td>546</td>
<td>407</td>
</tr>
</tbody>
</table>

Because of the relatively greater abundance of ribosomal aggregates in rat spleen, all subsequent experiments were performed in this species.

**Amino Acid Incorporation by Polyribosomes**—The role of polyribosomes in cellular protein synthesis was studied in the intact animal and in tissue fragments. Similar results were obtained with both techniques. The exposure to 14C-amino acids was brief to ensure predominant labeling of newly synthesized polypeptide chains and not ribosomal structural protein.

Table I shows that rat spleen polyribosomes prepared from tissue fragments became labeled after only 1 min of incubation and that the incorporation of amino acids into protein was proportional to the period of incubation.

The distribution of this radioactivity among the different ribosomal aggregates was studied by sucrose density gradient analysis. Fig. 3 shows the radioactivity patterns obtained after 1 and 5 min of incubation. Polyribosomes containing 2 mg of RNA were applied to each gradient, and the optical density patterns were identical (a broad hump similar to the radioactivity profile with a maximum in tube 6). Several peaks of radioactivity were observed, most evident in the preparation incubated for 5 min. These peaks appeared to correspond to the known position of monomers (arrow) and to the probable positions for dimers, trimers, tetramers, and heavier aggregates.

A distinct monomer peak of radioactivity was observed after 5

![RAT SPLEEN "MICROSOMES"](image-url)

**Fig. 5.** Approximately 2 g (wet weight) of rat spleen fragments were incubated with 14C-lysine and 14C-isoleucine for 10 min. After homogenization, the postmitochondrial supernatant fraction was prepared and centrifuged at 40,000 rpm for 2 hours in a Spinco 40 rotor. The microsomal pellet thus obtained was suspended in Medium A, and an aliquot was added to a 10 to 34% linear sucrose gradient (see "Experimental Procedure") prepared on top of a 5-ml layer of 68% sucrose. Centrifugation was for 1 hour at 25,000 rpm in an SW 25.1 rotor. The prominent peak in tube 7 is at the interface between the 68 and 34% sucrose, and the arrow indicates the position of single ribosomes.
min of incubation but not after 1 min. In other experiments a major peak of radioactivity was sometimes found in the monomer region (e.g. see Fig. 4), particularly after longer exposure to radioactive amino acids (10 to 20 min). This radioactivity was not present in ribosomal structural protein (see the next section). It is probable that the newly synthesized protein in the monomer region represents polypeptide chains formed initially on polyribosomes which remain attached to single ribosomes when the latter leave the aggregate. A similar temporal sequence of polypeptide chain synthesis has been demonstrated in cell-free experiments in vitro with Escherichia coli (1) and peanut cotyledon (4) polyribosomes.

These experiments establish that ribosomal aggregates are the sites of amino acid incorporation in rat spleen. The next series of experiments shows that this incorporation occurs into nascent polypeptide chains.

**Nascent Polypeptide Chains on Polyribosomes**—As already shown, treatment of rat spleen polyribosomes with ribonuclease in vitro caused a breakdown of polyribosomes and an increase in single ribosomes. When labeled polyribosomes were so treated, there was a shift in the distribution of radioactivity from the heavier aggregates toward the smaller ribosomal aggregates and monomers. There was no increase of radioactivity at the top of the gradient, however, indicating that none of the labeled protein was rendered soluble by the ribonuclease treatment. This experiment shows that labeled protein remains intimately associated with ribosomes when polyribosomes are disrupted by hydrolysis of messenger RNA.

By contrast, the labeled protein is readily released by treatment in vitro of polyribosomes with puromycin. This inhibitor of protein synthesis is thought to cause the release of nascent polypeptide chains by interfering at the site of attachment of the soluble RNA to the ribosome (21-23). As shown in Fig. 4, puromycin treatment resulted in the release of 68% of the total radioactivity. This effect was distributed uniformly throughout the gradient, indicating that the majority of the label, including the prominent peak in the monomer region, is present in nascent polypeptide chains.

The rapid turnover of the newly synthesized protein was further demonstrated by a "chase" experiment in which the incorporation of radioactive lysine and isoleucine was specifically inhibited after 3 min by the introduction of a 200-fold excess of 

### Table III

**Conditions for cell-free amino acid incorporation**

<table>
<thead>
<tr>
<th>Condition</th>
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<tbody>
<tr>
<td>Complete system</td>
</tr>
<tr>
<td>Minus polyribosomes</td>
</tr>
<tr>
<td>Minus pH 5 fraction</td>
</tr>
<tr>
<td>Minus ATP, P-enolpyruvate, &quot;kinase&quot;</td>
</tr>
<tr>
<td>Plus ribonuclease, 5 μg.</td>
</tr>
<tr>
<td>Plus puromycin, 8 × 10⁻⁴ M</td>
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<table>
<thead>
<tr>
<th>cpm/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>305</td>
</tr>
<tr>
<td>33</td>
</tr>
<tr>
<td>102</td>
</tr>
<tr>
<td>35</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>47</td>
</tr>
</tbody>
</table>

This series of experiments indicates, therefore, that the amino acids rapidly incorporated by ribosomal aggregates in rat spleen are present in nascent polypeptide chains.

**Membrane-bound and Free Polyribosomes**—The yield of polyribosomes was almost identical whether or not the postmitochondrial supernatant fraction was treated with sodium deoxycholate prior to centrifugation through 2.0 M sucrose. This suggested that the majority of the polyribosomes in this fraction were free and not attached to membranes. Studies of 

\[ 1^4 \text{C} \] labeled rat spleen microsome fractions on a modified sucrose gradient permitted a separation of membrane-bound and free polyribosomes and revealed an abundance of the latter. As shown in Fig. 5, membrane-bound polyribosomes collected at the interface between the 68 and 34% sucrose. Free polyribosomes and single ribosomes were distributed in the gradient, and soluble material remained at the top. The curves of optical density and radioactivity were parallel except in the monomer region which showed a lower specific activity than did the heavier aggregates. Approximately 26% of the total radioactivity was present in the membrane-bound fraction, and 50% was associated with the free polyribosomes and single ribosomes. The A₂₆₀:₄₅₀ ratio was 1.8 to 1.9 in the monomer region and 1.4 to 1.5 in the interface region, reflecting the higher protein content of the membrane-bound polyribosomes. A similar experiment performed with rat liver showed only a single peak at the interface with no radioactivity present in the gradient itself. A comparable result was obtained with rat liver by Henshaw, Bojarski, and Hiatt (24). Treatment of the spleen microsome fraction with 1% sodium deoxycholate resulted in an almost complete disappearance of the 260 μm absorbing material and radioactivity in the interface region with an increase of both optical density and radioactivity in the free polyribosome and monomer regions. These experiments show that the majority of cytoplasmic polyribosomes in the spleen are not attached to membranes. By contrast, ribosomes sedimenting with the 800 × g and 27,000 × g pellets appear to be membrane-bound since they sediment through 2.0 M sucrose only after treatment with deoxycholate. As in the liver (10), they are present primarily as dimers.

**Cell-free Protein Synthesis**—Rat spleen polyribosomes were found to be very active in stimulating cell-free protein synthesis. As shown in Table III, amino acid incorporation was dependent upon polyribosomes (a small amount of incorporation was often observed with the pH 5 fraction alone, probably because of the presence of trace amounts of ribosomal aggregates). In the absence of pH 5 fraction, protein synthesis was only one-third that of a complete system, thus demonstrating a requirement for added soluble RNA and enzymes. Either rat liver or spleen could serve as the source of the pH 5 fraction. Incorporation was also dependent upon an energy-generating system (ATP, P-enolpyruvate, and phosphokinase) and was inhibited by both ribonuclease and puromycin (Table III).
Fig. 6. Incorporation of $^{14}$C-lysine as a function of magnesium concentration. Incubation was carried out for 30 min as described in "Experimental Procedure."

Fig. 7. Incorporation of $^{14}$C-serine as a function of time. Incubation was carried out as described in "Experimental Procedure."
As shown in Fig. 6, a typical magnesium concentration curve was obtained with rat spleen polyribosomes. Cell-free protein synthesis was stimulated by magnesium through a range of 1 to 7 mM, reached a plateau from about 7 to 11 mM, and was inhibited at higher magnesium concentrations. It is known that magnesium has several important functions in protein synthesis and influences the interaction of messenger RNA and ribosomes (25, 26).

The kinetics of cell-free protein synthesis is shown in Fig. 7. The rate of incorporation was linear for 20 to 30 min and then gradually declined. Similar kinetics has been observed with other cell-free systems. The decrease in synthesis has been attributed to a defect in the attachment of single ribosomes to polyribosomes (27).

The incorporating ability of these polyribosomes was reduced almost to zero by “preincubation” in a complete system (minus “C-amino acid) for 30 min. Such preincubated ribosomes responded very well to synthetic polyribonucleotides. Thus, a 5- to 10-fold stimulation of phenylalanine incorporation was regularly obtained with 100 µg of polyuridylic acid. This suggests that single ribosomes were liberated from the polyribosomes during the period of preincubation and that such “free” ribosomes responded to exogenous messenger RNA.

**DISCUSSION**

Polyribosomes are composed of ribosomes, messenger RNA, and polypeptide chains undergoing active synthesis. The properties of these ribosomal aggregates include (a) sedimentation in sucrose density gradient centrifugation as a structure considerably heavier than single ribosomes, (b) dissociation into single ribosomes after ribonuclease treatment, (c) ability to synthesize protein which can be “chased off” by 3H-amino acids or released by puromycin but not by ribonuclease, and (d) ability to support amino acid incorporation in a cell-free system in vitro.

Based upon these criteria, the presence of polyribosomes in rat spleen has been demonstrated. One may conclude, therefore, that the spleen contains ribosomal aggregates active in protein synthesis and similar in properties to polyribosomes studied in other biological systems (1–10).

The size of polyribosomes has been assumed to correlate directly with the size of messenger RNA molecules. In rat spleen, aggregates varying from dimers to greater than tetramers were regularly observed. However, the spleen is rich in ribonuclease, and it is therefore possible that these aggregates already represent partial breakdown products formed by the hydrolytic action of splenic ribonuclease on messenger RNA. This would appear to be true for rabbit spleen polyribosomes, for example, where a large monomer peak was frequently observed. In rat spleen, however, monomers were rare, and polyribosomes actively stimulated cell-free protein synthesis, an indication that they contained functional messenger RNA (particularly since inhibition did occur in the presence of exogenous ribonuclease). Nevertheless, without more direct studies, it seems imprudent to make any assumptions about the size of messenger RNA in the spleen.

Cell-free amino acid incorporation dependent upon spleen microsomes or ribosomes has been described by Wust and Novelli (28). Their system also requires an energy source, magnesium, and a supernatant fraction. Unlike the cell-free preparation described in this report, however, their system synthesized protein only with a supernatant prepared from rat liver and not with one from rat spleen.

The nature of the protein being synthesized by the spleen polyribosomes is obviously of great interest. Other workers have demonstrated antibodies present in microsomes (29) and γ-globulin present in ribosomes (30) prepared from lymph nodes of immunized rabbits.

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**REFERENCES**

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