The Effect of Various Treatments in Vitro and in Vivo on the Binding of ^{125}I-Labeled Anti-Rat Serum Albumin Fab′ to Rat Tissue Polyribosomes†

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ABSTRACT: A quantitative assay was developed to permit estimation of the relative amounts of albumin-synthesizing polyribosomes in rat liver. The polyribosomes synthesizing albumin were identified by their capacity to bind anti-RSA Fab′ radiolabeled with ^{125}I. The anti-RSA Fab′-binding sites occur on the nascent peptide chains attached to liver polyribosomes. These binding sites can be saturated by preincubation of the polyribosomes with large quantities of unlabeled anti-RSA Fab′. The iodinated antibody did not react with polyribosomes isolated from a tissue which does not synthesize rat serum albumin. Pretreatment of hepatic polyribosomes with bovine pancreatic ribonuclease resulted in a 42% enhancement of binding of anti-RSA Fab′. Pretreatment of these polyribosomes with detergents or various levels of Mg^{2+} did not significantly affect the specific binding of the iodinated antibody. Anti-RSA Fab′ associated preferentially with membrane-bound polyribosomes when compared with free polyribosomes following their isolation from animals maintained either on a 90% or a 0% protein diet fed ad libitum. Binding of anti-RSA Fab′ to each A_{260} unit of membrane-bound polyribosomes is from 2.4 to 27 times greater than to each A_{260} unit of free polyribosomes. However, each A_{260} unit of free polyribosomes was found to associate with 1.8 times more anti-RSA Fab′ when compared with the "loosely bound" subclass of membrane-bound polyribosomes. Each A_{260} unit of the "tightly bound" subclass of membrane-bound polyribosomes reacted with 4.3 times as much antibody as compared with free polyribosomes. Polyribosomes isolated from the livers of rats sacrificed 6 h after treatment with actinomycin D showed a 42% reduction in their capacity to bind anti-RSA Fab′. Polyribosomes from rats sacrificed 2 h after treatment with actinomycin D showed no reduction in binding capacity. Free polyribosomes from three Morris hepatomas were capable of binding anti-RSA Fab′ whereas the antibody would not associate with the membrane-bound polyribosomes of the same hepatomas. Thus the binding of ^{125}I-labeled Fab′ antibody molecules to polyribosomes is a useful technique for the subcellular localization of polyribosomes synthesizing specific proteins and for the estimation of the relative proportions of such polyribosomes.

In order to understand the molecular mechanisms involved in the regulation of protein synthesis in the eukaryotic cell, a necessary step is the identification, localization, and quantification of specific mRNA[1] templates. This can be achieved in part through the use of immunochemical techniques which permit indirect identification of specific mRNA templates as a consequence of immunological recognition of the nascent chains of polyribosome–mRNA complexes. It is well recognized that antibodies against completed proteins will react with the polyribosomes synthesizing those proteins (Warren and Peters, 1965; Cowie et al., 1961; Holme et al., 1971a,b; Palacios et al., 1972; Ikehara and Pitot, 1973). The binding of radiolabeled antibody molecules with polyribosomes had been used to study the qualitative aspects of the synthesis of specific proteins by polyribosomes (Taylor and Schimke, 1974; Ikehara and Pitot, 1973). However, there are relatively few reports on the use of quantitative assays involving the binding of radiolabeled antibodies to polyribosomes (Palmiter et al., 1972; Konijn et al., 1973).

The liver cell contains polyribosomes in its cytoplasm as well as polyribosomes bound to the endoplasmic reticulum. The latter polyribosomes (membrane-bound) have been considered to be involved preferentially in the synthesis of proteins destined for extracellular use, such as is the case for rat serum albumin. Free polyribosomes have been thought to be involved mainly in the synthesis of proteins intended for intracellular use.

In this paper we describe the development of a highly sensitive assay which permits estimation of the relative proportions of polyribosomes synthesizing a specific protein. Data are presented which will provide a basis for further investigations as to the possible role that the endoplasmic reticulum might play in the translational control of genetic expression.

Materials and Methods

Materials. Anti-rat serum albumin IgG[1] was purchased from the Calbiochem Co., San Diego, Calif. Crystalline bovine pancreatic ribonuclease, papain, and yeast RNA were obtained from the Sigma Chemical Co., St. Louis, Mo. Na^{125}I was obtained from New England Nuclear Corp., Boston, Mass.; sucrose (ribonuclease-free) was from Mann Research Labs., Inc., New York, N.Y., and Chloramine-T from Eastman

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[1] Abbreviations used are: RSA, rat serum albumin; Fab′, the monovalent antibody fragment obtained by papain digestion of the IgG molecule (see Porter, 1959); IgG, 7S immunoglobulin; tRNA, transfer ribonucleic acid; mRNA, messenger ribonucleic acid; DEAE, diethylaminoethyl; TKM, 20 mM Tris-HCl (pH 7.4)–25 mM KCl–5 mM $MgCl_2$; Tris, tris(hydroxymethyl)aminomethane.

Kodak Co., Rochester, N.Y. Carboxymethylcellulose was purchased from Bio-Rad Lab., Richmond, Calif., and DEAE Whatman cellulose from W & R Balston Ltd., Kent, England.

Animals. All experiments using animals were performed with 200–350-g male Holtzman strain (Holtzman Co., Madison, Wis.) or Buffalo strain rats. The Morris hepatomas, 5123C, 7800, and 7777 were carried by intramuscular implantations in male albino rats of the Buffalo strain. Except where indicated in the Results, the rats were maintained on a Purina chow diet fed ad libitum. The lighting in the rooms used for housing the rats was automatically controlled with the lights on from 6:00 a.m. until 6:00 p.m. and off from 6:00 p.m. to 6:00 a.m. For certain experiments, as is indicated in the results, rats were maintained on a 0% protein diet for 5 days or a 90% protein diet for 6–10 days. During the course of the experiments, the food cups were placed in the cages at 5:00 p.m. and the cups were removed at 8:00 a.m. The animals were not fed during the dark period preceding the day that they were sacrificed.

Immunochemical Techniques. The IgG fraction of the sera was prepared by ion-exchange chromatography and ammonium sulfate precipitation according to Fahey (1967). The specificity of the anti-rat serum albumin was tested by Ouchterlony double diffusion and by immunoelectrophoresis. Both techniques indicated that the anti-rat serum albumin immunoglobulin was monospecific in reacting only with purified rat serum albumin.

Rat serum albumin was prepared as has been previously described (Debro et al., 1957) followed by chromatography on DEAE-cellulose with a linear phosphate (pH 7.6) gradient (0.01–0.15 M). Purified rat serum albumin when subjected to electrophoresis on sodium dodecyl sulfate–polyacrylamide gels (Cashman and Pitot, 1971) showed only a single protein band upon staining with Coomassie brilliant blue.

Quantitative immunoprecipitation reactions were carried out by adding a constant amount of anti-rat serum albumin IgG (0.5 mg) to conical, 3-ml, Pyrex centrifuge tubes containing varying amounts of purified rat serum albumin (vide supra). The reaction was allowed to proceed in 50 mM Na_2HPO_4 (pH 7.6) and 0.15 M NaCl in a final volume of 1.0 ml for 30 min at 37 °C followed by 18 h at 4 °C. Controls for nonspecific precipitation of protein were constructed by incubating nonimmune IgG (0.5 mg) with varying amounts of rat serum albumin. At the end of the incubation, mixtures were centrifuged at 183*g* in a Sorvall GLC-1 centrifuge at 4 °C for 20 min. The supernatant was removed by aspiration. The precipitates were washed twice with 0.5-ml aliquots of 50 mM Na_2HPO_4 (pH 7.6) in 0.15 M NaCl at 4 °C. The remaining supernatant washes were carefully removed following subsequent centrifugations and the protein content of the precipitates was determined. At the equivalence point 500 μg of anti-RSA IgG precipitated 9.5 μg of RSA.

For preparation of anti-RSA and nonimmune Fab′ products, the IgG molecules were hydrolyzed with papain according to the method of Porter (1959). IgG preparations (90–100 mg) in 2 ml of 0.1 M Na_2HPO_4 (pH 7.0)–0.01 M cysteine hydrochloride–2 mM Na_2 EDTA were incubated with papain (1 mg, 18 units of enzyme) for 7–18 h at 37 °C. The digested IgG was applied to a Sephadex G-25 column (2.0 × 35 cm) equilibrated with 0.1 M sodium acetate (pH 5.5) at room temperature. Those fractions (10 ml each) of the eluent having an absorbancy greater than 1.0 at 280 nm were pooled. This pooled eluent (50–60 ml) was applied to a carboxymethylcellulose column (2.5 × 35 cm) equilibrated with 0.1 M sodium acetate (pH 5.5) at room temperature. The proteins were eluted at room temperature with 0.1 M sodium acetate (pH 5.5) and were collected in 6-ml fractions at a flow rate between 20 and 100 ml/h. The absorbancy at 280 nm was measured using a Gilford spectrophotometer. Those fractions which constituted the majority of the eluent forming that peak of absorbancy which eluted first (see Results) were pooled. This eluent was adjusted to pH 7.2 with 6 M NaOH and made 65% saturated with ammonium sulfate. After 30 min at room temperature, the precipitate was collected by centrifugation at 7700*g* for 20 min in a Sorvall SS-34 rotor (Ivan Sorvall, Inc., Newtown, Conn.). The precipitate was solubilized in and dialyzed against 50 mM Na_2HPO_4 (pH 7.6) in 0.15 M NaCl. The final concentration of Fab′ was 2 mg/ml.

Two iodination procedures were used for radiolabeling the anti-RSA and nonimmune Fab′ preparations with ^{125}I. One method used was that of Sonoda and Schlamowitz (1970) which utilizes Chloramine-T as an oxidant. The second method, that of Palacios et al. (1972), requires the use of H_2O_2 as an oxidant and lactoperoxidase as a catalyst.

The Chloramine-T iodination procedure was performed in the following manner. To an Fab′ preparation (10 mg) in 2.0 ml of 50 mM Na_2HPO_4 (pH 7.0), in 0.15 M NaCl, was added 100 μCi of $Na^{125}I$ in 100 μl of the same buffer as the Fab′ preparation ($Na^{125}I$, carrier free, obtained from Amersham/Searle Corp., Arlington Heights, Ill.) at 4 °C. Freshly prepared Chloramine-T (667 μmol in 5.0 ml) was added over a 2-min period while the solution was stirred. The reaction mixture was incubated at 4 °C with stirring for 30 min. The Chloramine-T was neutralized with 667 μmol of $NaHSO_3$ in 1.2 ml of 50 mM Na_2HPO_4 (pH 7.0)–0.15 M NaCl. After 5 min of mixing, 1.0 M KI (0.2 ml) was added and the solution was passed through an AG 1-X8 anion-exchange resin (1.6 × 1.0 cm, 2.0-ml column) which had been equilibrated with 50 mM Na_2HPO_4 (pH 7.0)–0.15 M NaCl. The column was washed with 3 ml of the same buffer. The combined eluates were dialyzed at 4 °C against two to three changes (4 l. each) of 50 mM Na_2HPO_4 (pH 7.6) in 0.15 M NaCl. The specific activity of the final preparations varied from 10^6 to 10^7 cpm/mg protein.

The iodination procedure which utilizes lactoperoxidase and H_2O_2 was performed in a manner nearly identical with that described by Palacios (vide supra). To the Fab′ preparations (6 mg) in 2.0 ml of 50 mM Na_2HPO_4 (pH 7.0) in 0.15 M NaCl were added bovine milk lactoperoxidase (300 μg) (a generous gift from Dr. G. Mueller of this laboratory) and $Na^{125}I$ (1.2 mCi in 100 μl). Over a 2-min period with stirring, H_2O_2 (300 μl of a 0.1 mM solution) was added. After 30 min at room temperature with stirring, crystalline ammonium sulfate was added to the incubation mixture until the solution was 65% saturated with respect to ammonium sulfate. The precipitate that formed after a 30-min incubation at room temperature with stirring was collected by centrifugation at 4320*g* for 20 min in a Sorvall SS-34 rotor at 4 °C. The precipitate was dissolved in 1.0 ml of 50 mM Na_2HPO_4 (pH 7.6) in 0.15 M NaCl and washed through a DEAE-cellulose column (1.6 × 1.0 cm, 2.0 ml bed volume) equilibrated with the same buffer. The column was washed with 3.0 ml of 50 mM Na_2HPO_4 (pH 7.6) in 0.15 M NaCl and the combined eluents were dialyzed 18–24 h at 4 °C against two changes of 50 mM Na_2HPO_4 (pH 7.6) in 0.15 M NaCl. The specific activity of the final preparations was 0.5–1.0 × 10^8 cpm/mg protein. Despite the use of seemingly identical conditions, on several different occasions the iodination of Fab′ preparations in reactions catalyzed by Chloramine-T resulted in denaturation of the antibody. Loss of antibody reactivity did not occur fol-

lowing the iodination procedure wherein lactoperoxidase and H_2O_2 were used. For this reason the lactoperoxidase-catalyzed reaction was considered the preferred procedure for iodination of antibody preparations.

Preparation of Liver and Kidney Polyribosomes. Male Holtzman rats maintained on a 90% protein diet fed ad libitum for 6–10 days were decapitated and the livers and kidneys rapidly removed and chilled in double-distilled water at 4 °C. All subsequent procedures were performed at 4 °C. The organs were weighed, minced in 2 volumes of 0.44 M sucrose in TKM, and homogenized with ten strokes in a Potter-Elvehjem homogenizer. A postmitochondrial supernatant was prepared by centrifuging the homogenate for 10 min at 17 000*g* in an SS-34 Sorvall rotor. The postmitochondrial supernatant was treated with sodium deoxycholate (1.0% w/v) and then adjusted to 1.3 M sucrose in TKM (20 mM Tris-HCl (pH 7.4)–25 mM KCl–5 mM $MgCl_2$) by the addition of 1.47 volumes of 2.0 M sucrose in TKM. A 17-ml portion of this supernatant containing deoxycholate was layered over 10 ml of 2.0 M sucrose containing 0.5 M NH_4Cl in TKM. This discontinuous gradient was centrifuged at 123 000*g* maximum in polycarbonate tubes in a Beckman Type 42.1 rotor for 6 h at 4 °C. The surfaces of the pellets were washed several times with 50 mM Tris-HCl (pH 7.4). The polysomes were homogenized in the washing buffer and stored at −20 °C for as long as 2 months. Unless stated otherwise, all polysome preparations were treated with bovine pancreatic RNase (3 μg per ml of polysomal solution) at 4 °C for 30 min before being used in the assay for the determination of ^{125}I-labeled Fab′ binding (vide infra).

Preparation of Free and Membrane-Bound Polysomes. Polysomes were prepared from postmitochondrial supernatants (vide supra) as described by Ikehara and Pitot (1973). A 20-ml portion of the postmitochondrial fraction adjusted to 1.35 M sucrose in TKM was layered over 10 ml of 2.0 M sucrose in TKM and overlaid with 4 ml of 0.44 M sucrose in TKM. This discontinuous gradient was centrifuged at 123 000*g* maximum for 6 h in a 42.1 Beckman rotor at 4 °C. The rough endoplasmic reticulum which floated at the boundary between the 2.0 and 1.35 M sucrose layers was collected after aspiration of the solution above the rough endoplasmic reticulum. The 2.0 M sucrose in TKM which remained was decanted. The tubes containing pellets of free polysomes were rinsed three times with TKM. The solution containing the rough endoplasmic reticulum was diluted with 1 volume of TKM and was treated with sodium deoxycholate (1.0% w/v) like the total polysome preparations (vide supra). Both isolated free and membrane-bound polysomes were homogenized in a small volume (1–2 ml) of TKM. The suspension of polysomes was layered over 15–20 ml of 0.44 M sucrose in TKM–0.5 M NH_4Cl and centrifuged in a 42.1 rotor for 2 h. The pellets were rehomogenized in 50 mM Tris-HCl (pH 7.4) and clarified by centrifugation at 183*g* for 15 min. The polysome solutions (70–250 A_{260} units/ml) were stored for as long as 2 months at −20 °C.

For the preparation of the free, and of the loosely and tightly membrane-bound polyribosomes (Rosbash and Penman, 1971a,b), the modified method of Blobel and Potter (1967a) described by Ragland et al. (1971) and Tanaka and Ogata (1972) was employed. A postmitochondrial supernatant was obtained as for total polysome preparations and the free polyribosomes and rough endoplasmic reticulum were isolated from the postmitochondrial supernatant as is described above in the procedure utilized for the isolation of free and membrane-bound polyribosomes. The suspension of rough endoplasmic reticulum was diluted by the addition of 2 volumes of TKM. Bovine pancreatic ribonuclease was added to a final concentration of 3 μg/ml. The membrane solution was layered on 10 ml of 2.0 M sucrose in TKM and subjected to centrifugation for 16–18 h at 123 000*g* maximum in a Beckman 42.1 rotor. The loosely membrane-bound polyribosomes were pelleted during this centrifugation. The RNase-treated rough endoplasmic reticulum was located on the top of the 2.0 M sucrose. The polyribosomes and the rough endoplasmic reticulum were collected as described above. The solution containing the RNase-treated rough endoplasmic reticulum was diluted by the addition of 2 volumes of TKM and then solubilized in 1% (w/v) sodium deoxycholate. The tightly membrane-bound polyribosomes are released by this treatment and were collected by pelleting them through 2 M sucrose in TKM as is described above for the isolation of the other two classes of polyribosomes. The polyribosome pellets for all three classes were solubilized, washed with 0.5 M NH_4Cl, treated with RNase, and stored as is described in the preparation of free and membrane-bound polyribosomes.

^{125}I-Labeled Fab′ Binding to Polyribosomes. From 0 to 45 A_{260} units of polyribosomes in a constant volume of 50 mM Tris-HCl (varying between experiments from 0.1 to 2.0 ml) were incubated with ^{125}I-labeled Fab′ preparations (0.2–1.0 × 10^6 cpm) for either 1 or 18 h at 4 °C. The incubated samples were then layered over 0.44 M sucrose in TKM containing 0.5 M NH_4Cl or a discontinuous gradient of 0.25 M sucrose in TKM–0.5 M NH_4Cl–Triton 0.02% (w/v) layered over 1.0 M sucrose in TKM. The samples applied to the sucrose solutions were centrifuged in a 50 Ti rotor at 185 000*g* and 4 °C for 2 h with the use of 0.44 M sucrose, or for 4 h when the discontinuous sucrose gradient was used. The recovery of polyribosomes varied between 60 and 75% of the applied sample. Prolonged centrifugation did not increase the percent recovery. The solution above the pellet was decanted, the walls and the bottom of the tubes were wiped, and the pellet was solubilized in 0.5 ml of 50 mM Tris-HCl (pH 7.4) at room temperature. The solubilized pellet was transferred to a conical centrifuge tube to which was added an additional 0.7 ml of 50 mM Tris-HCl. The solution was centrifuged for 20 min at 183*g* in the GLC-1 Sorvall centrifuge. One milliliter of the solution was removed for determination of absorbancy at 260 nm using a Gilford spectrophotometer and of radioactivity using an Amersham/Searle γ counter (Amersham/Searle Corp., Arlington Hts., Ill.). The γ counter had an efficiency of 75% for ^{125}I.

Analytical Methods. Protein was determined by the method of Lowry et al. (1951), with bovine serum albumin as a standard. The absorbancy at 260 nm was used as a measure of the quantity of polyribosomes in the assay of ^{125}I-labeled Fab′ binding to polyribosomes. One A_{260} unit was defined as that quantity of polyribosomes contained in 1.0 ml of solution having an absorbancy of 1.0 as measured at 260 nm with a 1.0-cm light path.

Results

Isolation of the Fab′ Component from a Papain Digest of an IgG Preparation. It has been shown that much of the nonspecific interaction of the IgG molecule with polyribosomes when used in reactions between antibodies and polysomes is eliminated by use of the Fab′ protein of the IgG molecule (Holme et al., 1971a,b; Ikehara and Pitot, 1973).

Porter (1959) showed that the papain digest of rabbit γ globulin upon chromatographic separation on carboxymethylcellulose eluted as three peaks. The two peaks observed by Porter (vide supra) which appeared nearest to the solvent front contained the Fab′ portions of the IgG molecule. For the

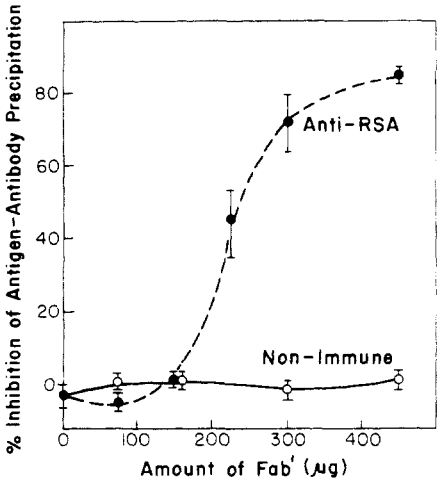

FIGURE 1: Inhibition of immunoprecipitation by Fab' proteins. Anti-rat serum albumin Fab', nonimmune rabbit Fab', and anti-rat serum albumin IgG were prepared as described under Materials and Methods. Rat serum albumin (RSA) (6.2 μg) was incubated with increasing amounts of anti-RSA Fab' or nonimmune rabbit Fab' at 37 °C for 1 h. Then anti-RSA IgG was added at a level of antibody excess (375 μg). An additional incubation at 37 °C for 1 h and at 4 °C for 18 h preceded the sample preparation and protein determination as described in the Materials and Methods. Closed circles and open circles represent anti-RSA Fab' and nonimmune Fab', respectively.

purpose of recovering the combined Fab' components and to avoid precipitation of protein encountered upon use of 0.01 M sodium acetate (pH 5.5) (McLaughlin, unpublished data), the procedure of Porter (vide supra) was modified. Separation of the Fab' portion from the Fc portion of the papain-digested IgG molecule was achieved by the use of 0.1 M sodium acetate (pH 5.5) as the eluting buffer for the carboxymethylcellulose chromatography.

The elution profile of the components of papain-treated IgG preparation (data not shown) revealed one major peak identified as the Fab' portion and one minor peak which is tentatively identified as the Fc portion of the IgG molecule. The component of the minor peak was not further characterized. The protein contained in pooled fractions of the major peak upon analysis by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate revealed an absence of an intact heavy chain and presence of two Coomassie-blue-stained proteins with estimated molecular weights of 31 000 and 26 000. These molecular weights are approximately what would be expected for the papain-cleaved heavy chain and the intact light chain of the Fab' molecule, respectively (Porter, 1959; Marler and Tanford, 1963).

Binding of Fab' Preparations to Rat Serum Albumin. It has been shown (Nisonoff et al., 1960) that preincubation of an antigen with an Fab' preparation inhibits the immunoprecipitation of that antigen by the IgG antibody from which the Fab' molecule was obtained. A similar effect was noted upon incubation of varying amounts of anti-RSA Fab' with RSA (6.2 μg) at 37 °C for 1 h followed by the addition of anti-RSA IgG (375 μg) and a second incubation at 37 °C for 1 h. The quantitative levels of inhibition of immunoprecipitation achieved with the use of increasing amounts of anti-RSA Fab' are presented in Figure 1. The precipitate measured in the nonimmune control tubes was averaged (50 μg/ml) and used as the 0% value for calculation of percent inhibition. It is to be noted that the use of increasing amounts of nonimmune Fab' did not inhibit immunoprecipitation of RSA by anti-RSA IgG. Therefore, the biological activity of the Fab' molecule before and after iodination could be tested by its ability to inhibit immunoprecipitation of rat serum albumin by anti-rat serum albumin IgG (Figure 1). This inhibition of precipitation could be demonstrated by direct visual examination as well as by quantitative determination of the protein precipitated. The ability of the anti-rat serum albumin Fab' to inhibit precipitation of rat serum albumin by anti-rat serum albumin IgG is characteristic of a monovalent antibody molecule (Porter, 1959; Nisonoff et al., 1960). It is seen (Figure 1) that the inhibition of immunoprecipitation is characterized by a sigmoidal curve of percent inhibition vs. amount of inhibitor added. This sigmoidal function could be a consequence of the cooperative effect of the binding of Fab' molecules resulting in steric hindrance of the reaction of the IgG molecules with antigenic determinants.

Binding of ^{125}I-Labeled Anti-RSA Fab' to Polyribosomes. In order to assess the specificity of the binding of ^{125}I-labeled anti-RSA Fab' to ribosomes involved in the synthesis of RSA, kidney and liver polyribosomes were incubated with ^{125}I-labeled anti-RSA Fab' and the level of Fab' binding was determined as described in the Materials and Methods. Polyribosomes from the kidney (an organ which does not synthesize rat serum albumin) (Rothschild et al., 1972), after exposure to isolated rat serum albumin during isolation, do not bind ^{125}I-labeled anti-RSA Fab' molecules. Liver polyribosomes isolated under similar circumstances are capable of binding ^{125}I-labeled anti-RSA Fab' molecules in a quantitative manner (data not shown).

It has been reported (Konijn et al., 1973; Palmiter et al., 1972) that incubation of ^{125}I-radiolabeled antibody with polyribosomes followed by pelleting of those immunoreacted polyribosomes through sucrose gradients permits one to establish a linear relationship between the level of radioactivity associated with the polyribosomal pellet and the amount of polyribosomes added to the incubation mixture. Controls for the nonspecific association of radioactivity with the ribosomal pellets were not used in those studies. A suitable control for this nonspecific cosedimentation of radioactivity would be the use of ^{125}I-labeled nonimmune antibody immunoglobulin preparations. The use of such a control in the determination of binding of ^{125}I-labeled anti-RSA Fab' molecules to liver polyribosomes is demonstrated in Figure 2. In this experiment two levels of ^{125}I-labeled Fab' preparations were incubated at 4°C for 18 h with varying amounts of polyribosomes. In Figure 2A a relatively high level of ^{125}I-labeled Fab' was utilized whereas in Figure 2B a relatively low level was used (see Legend of Figure 2 and Materials and Methods for experimental details). It is important to note that under the assay conditions detailed in the legend for Figure 2A, wherein a high level of ^{125}I-labeled Fab' preparation was used, the binding of the ^{125}I-labeled anti-RSA Fab' to polyribosomes is linear throughout the range of polyribosomes utilized. That is, the polyribosomes are the limiting component in this antigen-antibody reaction. However, in the situation where a low level of ^{125}I-labeled Fab' is used (Figure 2B), the linear relationship between binding of anti-RSA Fab' to polyribosomes exists between 0 and 2.0 A_{260} units whereas the ^{125}I-labeled anti-RSA Fab' component becomes a limiting reactant when incubated with greater than 2.0 A_{260} units of polyribosomes. The amount of nonspecific cosedimentation of radioactivity with pelleted polyribosomes preincubated with radiolabeled antibody Fab' preparations can be ascertained through the use of ^{125}I-labeled nonimmune Fab' preparations as controls. The validity of the use of this type of control is supported by the observation that subtraction of appropriate nonimmune control

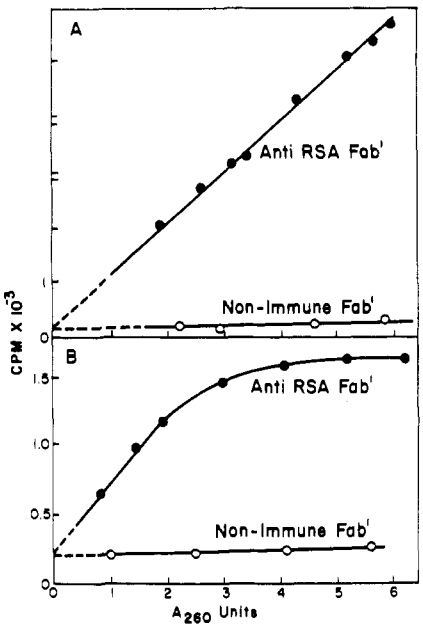

FIGURE 2: The effect of the incubation of a high level (A) or a low level (B) of ^{125}I-labeled Fab' preparations on the binding of ^{125}I-labeled Fab' molecules to hepatic polyribosomes. Increasing amounts of total liver polyribosomes were incubated at 4 °C for 18 h with 675 000 cpm of ^{125}I-labeled Fab' preparations per assay in A or with 200 000 cpm of [^{125}I]Fab' preparations per assay in B. See the Materials and Methods for further experimental details. The closed and open circles indicate the determinations of radioactivity and absorbancy at 260 nm for the anti-rat serum albumin Fab' and nonimmune Fab' preparations, respectively.

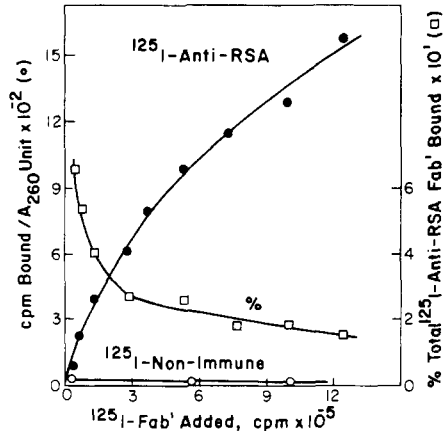

FIGURE 3: Binding of ^{125}I-labeled Fab' proteins to rat liver polyribosomes. Liver, total polyribosomes, ^{125}I-labeled anti-rat serum albumin (RSA) Fab', and ^{125}I-labeled nonimmunized rabbit Fab' were prepared as described in the Materials and Methods. Polyribosomes (2.4 A_{260} units) were incubated at 4 °C for 18 h with varying amount of the ^{125}I-labeled Fab' preparations. The aliquots were processed as described in Materials and Methods. The percentage of total anti-RSA Fab' bound is indicated by open squares. The binding of anti-RSA and nonimmune Fab' is shown by closed and open circles, respectively.

radioactivity values from the values for anti-rat serum albumin Fab' binding to polyribosomes permits extrapolation of the latter values to zero (Figure 2).

To ascertain whether or not the reaction of the ^{125}I-labeled anti-RSA Fab' preparations with polyribosomes had gone essentially to completion in a 1-h incubation, the effect of 18 h vs. 1 h of incubation on the binding of the antibody to liver polyribosomes was determined (Table I). When one compares the mean values for the binding of ^{125}I-labeled anti-RSA Fab' to polyribosomes following 18 h of incubation with the mean values for the binding following 1 h of incubation there is a 20 and 12% lower level of binding at the shorter time in experiments A and B, respectively. Incubation of ^{125}I-labeled Fab' preparations for an 18-h period was considered necessary in situations when maximum levels of binding were desired. However, in many subsequent experiments either 1 or 18 h of incubation of ^{125}I-labeled Fab' preparations with polyribosomes was considered to be a satisfactory period of incubation. The time of incubation was kept constant within individual experiments (see the legends of each figure and footnotes of each table for times of incubation used).

In Figure 3, a constant amount of polyribosomes was incubated at 4 °C for 18 h with increasing amounts of ^{125}I-labeled Fab' preparations in order to establish the characteristics of the reaction of the ^{125}I-labeled anti-RSA Fab' molecule with the polyribosomes under circumstances wherein the antibody molecules might saturate the binding sites on the nascent chains attached to the polyribosomes. A similar experiment was described by Palmiter et al. (1972) for the binding of ^{125}I-labeled antiovalbumin IgG to polyribosomes. As was the case in the ovalbumin system, the binding of the ^{125}I-labeled anti-RSA molecule to the polyribosomes increases rapidly for the samples containing increasing amounts of relatively low levels of the ^{125}I-labeled Fab' preparations. Over a range of

TABLE I: The Effect of Time of Incubation on the Binding of ^{125}I-Labeled Anti-RSA Fab' to Rat Liver Polyribosomes.[a]

Expt	Incubation Time (h)	^{125}I-Labeled Fab' Binding (cpm/A_{260})
A	1	579 ± 160
	18	812 ± 15
B	1	3614 ± 209
	18	4102 ± 507

[a] Total liver ribosomes were prepared (see Materials and Methods) and incubated with ^{125}I-labeled anti-RSA Fab' or ^{125}I-labeled nonimmune Fab' (control) at 4 °C. Aliquots in quadruplicate were processed as described in the Materials and Methods in order to determine the level of binding. The mean of four observations ± the standard deviations for anti-RSA binding minus nonimmune control values are shown. The Fab' preparations used in the experiments A and B were iodinated using the catalysts Chloramine-T and lactoperoxidase, respectively.

40-fold of the amount of ^{125}I-labeled Fab' added, the binding sites on the nascent chains attached to the polyribosomes were not saturated and the amount of binding of ^{125}I-labeled anti-RSA Fab' continued to rise. Conversely the percentage of the total ^{125}I-labeled anti-RSA Fab' added which binds to the polyribosomes decreases rapidly at low concentrations of Fab' and more slowly at higher Fab' concentrations. The sites of interaction of the ^{125}I-labeled anti-RSA Fab' molecule with the liver polyribosomes have been reported to be the antigenic determinants of the nascent chains for rat serum albumin (Ikehara and Pitot, 1973).

The Effects of Various Treatments on the Binding of ^{125}I-Labeled Fab' Preparations to Polyribosomes. As a means of assessing the specificity of the binding of ^{125}I-labeled anti-RSA Fab' molecules to polyribosomes, unlabeled Fab' preparations were preincubated with polyribosomes which were subsequently incubated with ^{125}I-labeled anti-RSA Fab' preparations (see Table II). As shown in Table II the preincubation of polyribosomes with an unlabeled anti-RSA Fab'

TABLE II: The Effect of Unlabeled Fab′ Preparations on the Binding of ^{125}I-Labeled Fab′ Preparations to Liver Polyribosomes.[a]

Unlabeled Fab′	^{125}I-Labeled Fab′	cpm/A_{260}
	Anti-RSA	5047 ± 507
	Nonimmune	945 ± 83
Anti-RSA	Anti-RSA	746 ± 34
Nonimmune	Anti-RSA	4340 ± 123

[a] The preparation of liver total ribosomes and Fab′ proteins and the iodination procedure using lactoperoxidase as a catalyst have been described under the Materials and Methods. Aliquots in triplicate of ribosomes (10 A_{260} units) were incubated with unlabeled Fab′ preparations (0.4 μg) at 4 °C for 1 h. The ^{125}I-labeled Fab′ protein was then added (600 000 cpm, 6 μg of protein) for an additional incubation at 4 °C for 18 h. The level of binding was determined (see Materials and Methods) and is presented as the mean of three observations.

preparation, but not with an unlabeled nonimmune Fab′ preparation, resulted in complete blockage of the binding of the ^{125}I-labeled anti-RSA Fab′ molecule to the polyribosomes. It is to be noted that the amounts of unlabeled Fab′ preparations (400 μg) added were considerably in excess when compared with the amounts of radiolabeled Fab′ preparations (6 μg). Similar data, qualitative in nature, have been reported by Gonzalez et al. (1974) and by Taylor and Schimke (1974). These authors found that preincubation of polyribosomes of the hen oviduct and rat liver with unlabeled IgG could block the subsequent binding of ^{125}I-labeled antibody molecules to the preincubated polyribosomes.

Considerable evidence that the structure and function of the polyribosome is dependent upon Mg^{2+} exists (Weiss and Morris, 1973). Therefore, in our studies it was of importance to determine the effect of magnesium in the incubation medium on the binding of ^{125}I-labeled Fab′ preparations to polyribosomes. No significant effect of magnesium ion on the binding of the radiolabeled Fab′ preparations to ribosomes was observed over a concentration range of 0 to 17 mM which includes levels of Mg^{2+} sufficient to cause precipitation of polyribosomes (Palmiter, 1974). Because of the precipitation of significant proportions of ammonium chloride washed polyribosomes after freezing and thawing (McLaughlin, unpublished observations), magnesium salts were omitted from buffers used for the storage of polyribosomes and from buffers used in the determination of the binding of ^{125}I-labeled Fab′ preparations to polyribosomes (see Materials and Methods).

In order to determine the optimum conditions of ^{125}I-labeled anti-RSA Fab′ binding to polyribosomes, experiments were conducted to examine the effect of the presence of ammonium chloride and Triton X-100 in the gradients used for separation of the unreacted ^{125}I-labeled anti-RSA Fab′ molecules from the ^{125}I-labeled anti-RSA Fab′ molecules complexed with polyribosomes (see the Materials and Methods). The addition of either ammonium chloride or of Triton X-100 to the gradients results in reduction of the nonspecific radioactivity cosedimenting with the polyribosomes (data not shown). Since the isolation of free and membrane-bound polyribosomes normally involves detergent treatment of the membrane-bound polyribosomes, it was important to establish whether or not this differential detergent treatment of free and membrane-bound polyribosomes might affect the binding of ^{125}I-labeled Fab′ preparations to polyribosomes. The treatment of free polyribosomes with either Triton X-100 or deoxycholate did not significantly affect the binding of ^{125}I-labeled anti-RSA Fab′ to those polyribosomes (data not shown).

Treatment of rat liver polyribosomes with bovine pancreatic ribonuclease resulted in a 42% enhancement of the binding of anti-RSA Fab′ molecules to ribonuclease-treated polyribosomes when compared with Fab′ binding to untreated polyribosomes (data not shown). To obviate an artifactual increase in binding due to variable degradation of polyribosome preparations by RNase, all subsequent polyribosome preparations were treated with RNase before being used in the reaction with ^{125}I-labeled Fab′ molecules (see the Materials and Methods).

The Effects of Treatments in Vivo on the Binding of ^{125}I-Labeled Anti-RSA Fab′ Preparations to Free and Membrane-Bound Liver Polyribosomes. The experiment described in the legend to Table III was conducted to ascertain whether or not modulation of the intake of dietary protein would affect the relative binding of ^{125}I-labeled anti-RSA Fab′ preparations to membrane-bound and free hepatic polyribosomes. Although there is some variation in the binding of ^{125}I-labeled anti-RSA Fab′ molecules (1.0×10^6 cpm) to membrane-bound and free polyribosomes, in all four experiments following incubation at 4 °C for 1 h, the membrane-bound polyribosomes were found to bind preferentially ^{125}I-labeled anti-RSA Fab′ preparations as compared with free polyribosomes. This preferential binding is independent of the level of protein in the diet. Valid comparisons of the *absolute* values of binding of ^{125}I-labeled anti-RSA Fab′ to polyribosomes in the experiments depicted in Table III cannot be made since different preparations of iodinated anti-RSA Fab′ were utilized in the three experiments. The maintenance of rats on a 0% or 90% protein diet fed ad libitum does result in changes in the synthesis of rat liver serine dehydratase on free and membrane-bound polyribosomes (McLaughlin and Pitot, 1976).

An increase in the synthesis of ovalbumin relative to total protein synthesis occurred upon treatment of chick oviduct magnum explants with actinomycin D (Palmiter and Schimke, 1973). These authors proposed that inhibition of RNA synthesis by administration of actinomycin D resulted in an increase in the proportion of long-lived mRNAs such as the mRNA for ovalbumin as compared with total mRNA population. Thus the mRNA for ovalbumin could be translated at an increased rate because of more favorable competition for factors which were rate limiting in the processes of translation. If a similar mechanism is operational in rat liver, one would expect the binding of ^{125}I-labeled anti-RSA Fab′ to polyribosomes from the livers of rats treated with actinomycin D to be greater than the binding of the radiolabeled antibody to polyribosomes from untreated controls. Under the experimental conditions described in the legend to Figure 4, the binding of ^{125}I-labeled anti-RSA Fab′ molecules (10^6 cpm) to total liver ribosomes from animals treated with actinomycin D for 2 h is not affected, whereas the binding of ^{125}I-labeled anti-RSA Fab′ to polyribosomes from animals treated with actinomycin D for 6 h is significantly reduced.

It is conceivable that the "superinduction" of albumin synthesis would only be observed in studies wherein the longer term effects of actinomycin D were examined. The reduction in binding of the antibody could reflect a transcriptional effect of actinomycin D whereby mRNA synthesis is blocked and albumin synthesis decays as a function of the half-life of the mRNA coding for albumin. A 42% reduction in binding capacity of the antibody to polyribosomes isolated 6 h after actinomycin D administration permits an estimate of the ap-

TABLE III: Binding of ^{125}I-Labeled Anti-RSA Fab' to Free and Membrane-Bound Polyribosomes from Livers of Rats Fed a 0% or 90% Protein Diet.[a]

Expt	Dietary Protein (%)	Polyribosome Class	Binding of ^{125}I-Labeled Anti-RSA Fab' cpm/A_{260}	Ratio of Binding of Bound Polyribosomes to Free Polyribosomes
I	90	Membrane-bound	2700 ± 150	19
		Free	144 ± 30	
II	90	Membrane-bound	1513 ± 274	18
		Free	85 ± 50	
III	0	Membrane-bound	6473 ± 1080	27
		Free	242 ± 130	
IV	0	Membrane-bound	1600 ± 200	16
		Free	100 ± 40	

[a] Isolated ribosomes were incubated with ^{125}I-labeled anti-RSA Fab' or ^{125}I-labeled nonimmune Fab' (1.0×10^6 cpm) at 4 °C for 1 h. The ^{125}I-labeled Fab' preparations were prepared using lactoperoxidase as a catalyst. The values for the level of binding of ^{125}I-labeled anti-RSA Fab' minus the values found using the appropriate ^{125}I-labeled nonimmune Fab' controls are presented as the means ± the standard deviations. There were five to eight observations per group. The values of binding of ^{125}I-labeled anti-RSA Fab' to membrane-bound ribosomes are divided by the values of binding to free ribosomes for calculation of the ratio of binding.

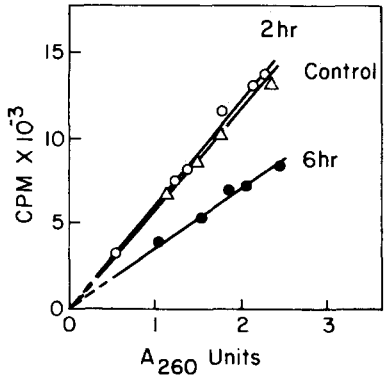

FIGURE 4: The effect of antinomycin D administered in vivo on binding of ^{125}I-labeled anti-RSA Fab' to hepatic polyribosomes. Actinomycin D was administered intraperitoneally (2 mg/kg body weight) to six rats. Three of these rats were sacrificed at 2 h, and the remaining three rats at 6 h after the administration of actinomycin D. A third group of three rats serving as controls were not treated. Liver total polyribosomes were isolated (see Materials and Methods). The binding of ^{125}I-labeled anti-RSA Fab' to the polyribosomes from the three groups of animals was determined as is described in Materials and Methods. The values presented for ^{125}I-labeled anti-RSA Fab' binding have been corrected for ^{125}I-labeled nonimmune Fab' control values. The open triangles represent the control animal group. The 2-h post-actinomycin D administration and the 6-h post-actinomycin D administration groups are represented as open and closed circles, respectively.

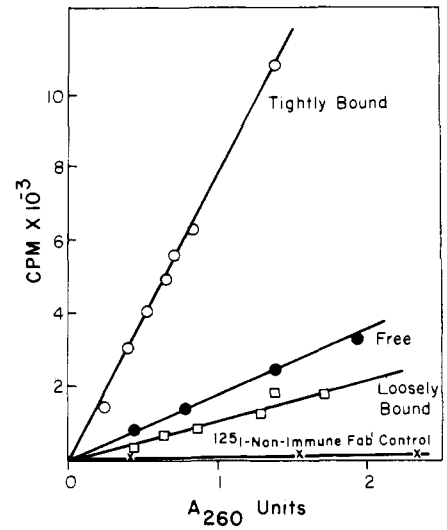

FIGURE 5: The binding of ^{125}I-Fab' preparations to free and to "loose" and "tight" membrane-bound polyribosomes of rat liver. Free polyribosomes and tightly membrane-bound polyribosomes were isolated as is described in the Materials and Methods. The binding of the ^{125}I-labeled Fab' preparations to the isolated polyribosomes was then determined (see Materials and Methods). The values for the binding of ^{125}I-labeled anti-RSA Fab' to free (●), "loose" membrane-bound (□), and "tight" membrane-bound (○) polyribosomes are presented. The binding of ^{125}I-labeled nonimmune Fab' (X) to free polyribosomes is also seen in the figure.

proximate half-life of the synthesis of rat serum albumin of 8 h. This estimation assumes that actinomycin D has no effect on albumin synthesis at the translational level. However, Goldstein et al. (1974) have shown that as early as 2 h following actinomycin D treatment of cells in culture there occurs an inhibition of initiation of nascent chain formation. Therefore, an estimate of the half-life of synthesis of a specific protein such as rat serum albumin when based on reduction of synthesis of the protein following actinomycin D treatment is subject to error owing to the possible translational effects of the drug.

Membrane-bound polyribosomes have been reported to be composed of two subclasses ("loose" and "tight" polyribosomes) (Rosbash and Penman, 1971a,b) which are functionally different (Zauderer et al., 1973; Tanaka and Ogata, 1972). For the purpose of our studies, ribonuclease treatment of the rough endoplasmic reticulum isolated from rat liver was utilized to release the loosely membrane-bound polyribosomes. These loosely membrane-bound polyribosomes were found (Figure 5) to have the fewest relative number of polyribosomes synthesizing rat serum albumin as compared with the free or the tightly membrane-bound polyribosomes. The tightly membrane-bound polyribosomes were found to be the preferential sites of synthesis of rat serum albumin (Figure 5).

Uenoyama and Ono (1972) reported that free polyribosomes of the Morris hepatoma 5123D preferentially synthesized rat serum albumin when compared with the synthesis of this protein by the membrane-bound polyribosomes isolated from the same tumor. Figure 6 demonstrated that there is binding

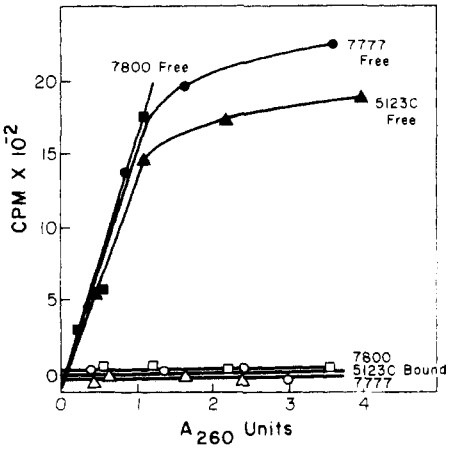

FIGURE 6: The binding of ^{125}I-labeled anti-rat serum albumin Fab' to free and membrane-bound polyribosomes isolated from Morris hepatomas. Animals bearing transplantable Morris hepatomas (see the Materials and Methods) were maintained on chow diets fed ad libitum. Free and membrane-bound polyribosomes were isolated as described in the Materials and Methods. The binding values for ^{125}I-labeled anti-RSA Fab' were determined and corrected for nonspecific cosedimentation of radioactivity by subtraction of the appropriate ^{125}I-labeled nonimmune control values. The closed squares, triangles, and circles represent the values for the free polyribosomes from the hepatomas 7800, 7777, and 5123C, respectively. The open symbols represent the values for the membrane-bound ribosomes from those hepatomas (see Figure 5 for further experimental details).

of ^{125}I-labeled anti-RSA to the free polyribosomes of the Morris hepatoma 5123C as well as to the free polyribosomes of the hepatomas 7777 and 7800. However, there is no binding of the ^{125}I-labeled anti-RSA Fab' molecule to the membrane-bound polyribosomes from these three hepatomas. Note that a linear relationship between the number of A_{260} units assayed and the level of radioactivity is observed only between 0.0 and 1.0 A_{260} unit. The reason for this phenomenon is not fully understood. This short range of linearity in the amount of ^{125}I-labeled Fab' binding to polyribosomes would not seem to be a consequence of the ^{125}I-labeled anti-RSA Fab' preparations being limiting in these assays since the same iodinated preparation when incubated with liver polyribosomes was capable of reacting and yielding linear binding characteristics to levels of at least 10 000 cpm (See Figure 5).

Discussion

The radioimmunochemical determination of antigenic protein which is polyribosome associated is dependent on a clear differentiation of specific from nonspecific interactions of radiolabeled antibody preparations with polyribosomes. The nonspecific interaction of intact IgG molecules with ribosomes (Holme et al., 1971a,b) and ribosomal subunits (Ikehara and Pitot, 1973) has been reported. Even in the case of the synthesis of rat serum albumin, which has been reported to consist of from 2 to 12% of total hepatic protein synthesis (Schreiber et al., 1969; Peters and Peters, 1972), the use of a radiolabeled IgG antibody would compromise the utility of a quantitative assay for polyribosomes synthesizing rat serum albumin. It should be noted that the use of ^{125}I-labeled anti-RSA IgG in the binding to rat liver polyribosomes has been reported (Taylor and Schimke, 1974). These authors described a qualitative assay for polyribosomes synthesizing rat serum albumin. They minimized the levels of nonspecific interaction partly by the use of antibody preparations that had been purified by affinity chromatography.

Another potential difficulty in the determination of specific antigens which are polyribosome associated would arise if a polyribosome preparation were contaminated with protein antigen present in the cytosol. Kidney polyribosomes exposed to rat liver cell supernatant containing rat serum albumin after washing by sedimentation through sucrose do not react with ^{125}I-labeled anti-RSA Fab'. This finding supports the contention that rat serum albumin did not contaminate washed liver polyribosomes. Furthermore, since kidney cells do not synthesize albumin, anti-RSA Fab' does not react with polyribosomes which are not involved in the synthesis of rat serum albumin. These data are consistent with the hypothesis that the anti-RSA Fab' binding site is located on the nascent chains of rat serum albumin which are attached to hepatic polyribosomes.

The basic principle involved in the quantitation of the specific polyribosomes synthesizing rat serum albumin is that the polyribosomes are added at levels such that they are the limiting reactant (Figure 2). Although it is possible to saturate the antigenic determinants or binding sites with antibody molecules (Table II), this requires relatively large quantities of antibody preparations. Hence, the use of an assay for quantitation of specific polyribosomes whereby saturating levels of radiolabeled antibody are used does not seem practical (Figure 2).

The binding of the anti-rat serum albumin antibody to polyribosomes under conditions where the polyribosomes are the limiting reactant permits one to make estimates and comparisons of the relative quantity of polyribosomes synthesizing rat serum albumin. At least three different circumstances in vivo would theoretically alter the values for radiolabeled antibody binding when expressed as the quantity of antibody bound per unit of polyribosomal material assayed (e.g., cpm of ^{125}I-labeled anti-RSA bound/per A_{260} unit of polyribosomes). One such circumstance would be an increase or decrease in the quantity of actively translating mRNA templates for rat serum albumin relative to all mRNA templates. Secondly, a variation in the number of ribosomes per unit length of rat serum albumin polyribosomes would affect the relative level of binding of the antibody. Thirdly, an increase or decrease in the amount of non-albumin-synthesizing polyribosomes without an attendant increase or decrease in the number of rat serum albumin-synthesizing polyribosomes would affect the relative binding of anti-rat serum albumin Fab'. Estimates of the relative amounts of actively translating polyribosomal units synthesizing a single protein can be made with the proviso that changes in antibody binding do not necessarily reflect changes in the quantity of translatable RNA for the antigen being studied. On the other hand, one circumstance pertinent to this discussion which would not alter the relative amount of binding of antibody to polyribosomes would be variations in the rate of translation, providing that such a variation did not result in changes in the number of ribosomes per unit length of polyribosomal mRNA.

Pretreatment of polyribosomes with ribonuclease caused an enhancement of the binding of the antibody. Although the mechanism of this response to ribonuclease treatment was not investigated, it is conceivable that formation of monomeric ribosomal units upon ribonuclease treatment of polyribosomes reduces steric hindrance which might exist in the interaction of the antibody with nascent chains attached to large polymeric ribosomal complexes. Palmiter et al. (1972) observed that there was little effect of ribonuclease treatment of avian oviduct polyribosomes on the binding of ^{125}I-labeled antiovalbumin IgG. It would appear from these data that either the potentially ribonuclease-sensitive phosphodiester bonds in the tRNA

portion of the peptidyl-tRNA molecule are protected by some ribosomal or polyribosomal component(s) or that this bond need not necessarily be intact to preserve the attachment of the peptidyl moiety to the ribosome in vitro.

Investigators have demonstrated that, although rat serum albumin is preferentially synthesized by membrane-bound polyribosomes, some of this protein is synthesized by free polyribosomes (Hicks et al., 1969; Redman, 1969; Ikehara and Pitot, 1973). Data presented in this paper (Table III and Figure 5) corroborate these findings. In addition, our data (Table III) demonstrate that considerable variation occurs in the capability of free polyribosomes to synthesize rat serum albumin. Still the majority of the in vivo synthesis of rat serum albumin occurs on membrane-bound polyribosomes. Assuming that 75% of the total hepatic polyribosomes are membrane bound and using the data for the binding of ^{125}I-labeled anti-RSA Fab' to free and membrane-bound polyribosomes (Table III), we estimate that membrane-bound polyribosomes are responsible for 88–99% of the total synthesis of rat serum albumin in vivo. Tanaka and Ogata (1972) reported that rat serum albumin was synthesized exclusively on the "tight" membrane-bound polyribosomes. Analysis of their data indicates, however, that some synthesis of albumin did occur on the "loose" type of membrane-associated polyribosomes. Data presented in Figure 5 generally corroborate their findings. However, we detected levels of synthesis of rat serum albumin occurring on free polyribosomes which were greater than those occurring on "loose" membrane-associated polyribosomes. Therefore, it is possible that the free polyribosomes of the rat liver are at times composed of variable quantities of polyribosomes involved in the synthesis of rat serum albumin. This variability expressed in vivo would in part explain the reported variation in relative ability of free polyribosomes to synthesize rat serum albumin as is seen in this paper (Figure 5 and Table III) and as has been observed by various investigators (Takagi and Ogata, 1968; Hicks et al., 1969; Redman, 1969; Ikehara and Pitot, 1973).

The proposal that membrane-bound polyribosomes are involved in the synthesis of proteins destined for extracellular use and that free polyribosomes preferentially synthesize proteins intended for intracellular use was supported by the findings of Uenoyama and Ono (1972). The data reported in this paper (Figure 6) show that the free polyribosomes of the three tumors Morris hepatomas 7800, 7777, and 5123C possess nascent rat serum albumin chains. The membrane-bound polyribosomes from these tumors do not exhibit nascent RSA chains. It is yet to be ascertained whether the Morris 7800 and 7777 tumors also fail to export albumin into the serum as has been observed in the case of the 5123 hepatoma.

The molecular mechanism which operates in the selection of specific polyribosomes for translation as a unit free in the cytoplasm or attached to the endoplasmic reticulum is of considerable debate (Adelman et al., 1973; Baglioni et al., 1971; Aronson, 1966; Blobel and Potter, 1967b; Rosbash, 1972; Pitot et al., 1974). The data showing that free hepatoma polyribosomes are the exclusive site of synthesis of rat serum albumin (Figure 6) suggest that the presence of actively translating templates may be required but is not sufficient for the functional association of a specific polyribosome with the endoplasmic reticulum.

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Hormonal and Nutritional Effects on the Binding of ^{125}I-Labeled Anti-Serine Dehydratase Fab′ to Rat Tissue Polysomes†

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ABSTRACT: With ^{125}I-labeled Fab′ specific for rat liver serine dehydratase it has been possible to localize polyribosomes synthesizing the enzyme under several different environmental conditions. Evidence is presented to show that, following the administration of amino acids in vivo, the relative synthetic capabilities of free and membrane-bound polyribosomes synthesizing serine dehydratase vary with time. Early during the period of induction of the enzyme by administration of amino acids or by feeding a high protein diet the majority of the newly synthesized enzyme is derived from membrane-bound polyribosomes. Later in the induction process an increasing proportion of the enzyme is synthesized by the free polyribosomes. Subcellular localization studies clearly show that serine dehydratase is synthesized by both subclasses of hepatic membrane-bound polyribosomes, the loose and tight membrane-bound polyribosomes, as well as by the free polyribosomes. It was found that the membrane-bound polyribosomes are the preferential sites of synthesis of the majority of serine dehydratase molecules in the Morris hepatomas 5123C and 7800. It is concluded that the synthesis of the enzyme, serine dehydratase, in rat liver is not discretely compartmentalized in either class of free or membrane-bound polyribosomes. Rather, the relative proportions of the serine dehydratase synthesizing polyribosomes within these two classes of polyribosomes can vary depending on the metabolic and physiologic state of the liver cell.

Considerable evidence (for recent review see Shires et al., 1974) has accumulated which demonstrates the presence of at least two distinct classes of polyribosomes in eukaryotic cells, those free in the cytoplasm and those attached to the endoplasmic reticulum. The membrane-bound polyribosomes have been proposed to be the sites of synthesis of proteins to be exported from the cell (Palade, 1956), whereas the free polyribosomes are considered to be preferentially involved in the synthesis of proteins destined for intracellular use (Birbeck and Mercer, 1961). However, exceptions to this generality have been reported in the case of two intracellular enzymes, NADPH[1]-cytochrome *c* reductase (Ragnotti et al., 1969) and serine dehydratase (Ikehara and Pitot, 1973), the synthesis of which has been shown to occur on both free and membrane-bound polyribosomes.

Identification of polyribosomes synthesizing a specific protein has been achieved by radioimmunochemical techniques which involve the binding of specific antibody molecules to polyribosomes (Warren and Peters, 1965; Taylor and Schimke, 1974; Ikehara and Pitot, 1973; Palacios et al., 1972; Konijn et al., 1973). Taking advantage of the sensitivity and specificity of this type of assay, we have investigated the intracellular localization of the synthesis of a specific intracellular enzyme, serine dehydratase, during modulation by various stimuli in vivo.

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[1] Abbreviations used are: SDH, serine dehydratase; Fab′, the monovalent antibody fragment obtained by papain digestion of the IgG molecule (see Porter, 1959); IgG, 7S immunoglobulin; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; NADH, reduced nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; EDTA, ethylenediaminetetraacetic acid; STKM, 0.44 M sucrose, 20 mM Tris-HCl (pH 7.4)–25 mM KCl–5 mM $MgCl_2$.