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Cell-Free Synthesis of Thyroglobulin*

Rejean Morais† and Irving H. Goldberg

ABSTRACT: Microsomal and polyribosomal fractions, prepared from calf thyroid glands, incorporate radioactive amino acid into both particle-bound and soluble proteins. In both cases, the incorporation of leucine into internal peptide linkage resembles that of protein synthesis in other mammalian systems. Sucrose density gradient centrifugation analysis shows the radioactive protein extractable from microsomes or ribosomes to sediment at 3–12 S, while a considerable portion of the labeled soluble protein released into the medium during incubation of microsomes but not of ribosomes resembles thyroglobulin in sedimentation and immunological properties. By the double-antibody technique, some of the labeled soluble protein sedimenting at 3–8 S was shown to be immunologically related to thyroglobulin and presumably subunits of thyroglobulin. These studies suggest a role for membrane-containing structures in the biosynthesis of thyroglobulin.

Thyroglobulin, a complex carbohydrate and iodine-containing protein with a sedimentation constant of 19 S and a molecular weight of 660,000, is the major protein synthesized in the thyroid gland. Previous reports from this laboratory and others (Seed and Goldberg, 1963, 1965a; Lissitzky *et al.*, 1965; Nunez *et al.*, 1965b) have shown that thyroid slices incorporate [^{14}C]amino acids into protein of one-quarter (3–8 S) and one-half (12 S) the size of thyroglobulin and that this isotope is "chased" by continued incubation with unlabeled amino acids from the smaller proteins into thyroglobulin-like material. The latter sediments at 17–18 S, is specifically precipitated with antithyroglobulin antibodies, and is converted to a material sedimenting at exactly 19 S by treatment with a chemical iodinating system (Goldberg and Seed, 1965; Nunez *et al.*, 1965b). These and other observations (Seed and Goldberg, 1965a; Sellin and Goldberg, 1965) have been interpreted to indicate

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that thyroglobulin subunits are synthesized on particulate structures and that iodination occurs primarily after aggregation of the subunits.

To gain insight into the mechanism of thyroglobulin synthesis, [^{14}C]amino acid incorporation was studied in cell-free preparations. The present paper reports some characteristics of microsomal and ribosomal systems derived from calf thyroid glands. It is shown that the microsomal system incorporates radioactive amino acid into protein which resembles thyroglobulin in sedimentation and immunological properties; the ribosomal system, however, fails to perform the same incorporation to any significant extent. These data suggest a role for membranous structures in the maturation of thyroglobulin whose subunits are synthesized on ribosomes. A preliminary report of portions of the work described here has been published (Morais and Goldberg, 1966).

Materials and Methods

Materials. L-[^{14}C]Leucine (200 μc/μmole) was purchased from New England Nuclear Corp. Puromycin was obtained from Nutritional Biochemical Corp. and PEP,[1] GTP, ATP, and pyruvate kinase were from California Corp. for Biochemical Research. Dr. H. Metzger generously supplied a sample of anti-bovine thyroglobulin antibody prepared in rabbits. Antisera to DNP-bovine serum albumin (Schlossman *et al.*, 1966) prepared from rabbits and to DNP-bovine plasma protein were a gift of Dr. S. Schlossman. Rabbit antiglobulin serum was obtained from Pentex Co.

Preparation of Subcellular Fractions. Fresh calf thyroid glands were obtained from a local abattoir within 30 min of slaughter and kept in ice-cold PBS solution. The glands were cleaned of fat and connective tissue and minced with a razor blade. The pulp obtained was suspended in ice-cold solution A (4 volumes/g), homogenized in a glass test tube with a loose-fitting Teflon pestle, and centrifuged at 10,000g for 15 min to yield a mitochondrial fraction. The postmitochondrial supernatant fraction was centrifuged at 105,000g for 1 hr to obtain a microsomal pellet and a supernatant fraction.

When polyribosomes were prepared, the postmitochondrial supernatant fraction was treated with 1% DOC (final concentration), layered onto 0.5 and 1.8 M sucrose solutions, as described by Wettstein *et al.* (1963), and centrifuged 2 hr at 105,000g. After centrifugation, the supernatant layers were decanted, the tubes were allowed to drain, and their inside walls were wiped dry with absorbant paper. The surface of the polyribosomal pellet was then washed twice with 0.5 ml of solution B.

Incubation Conditions. The microsomal pellet was resuspended by gentle homogenization in solution A and aliquots of about 9.0 mg of protein were added in incubation mixtures containing 40 μmoles of Tris-HCl (pH 7.8), 200 μmoles of sucrose, 70 μmoles of KCl, 15 μmoles of $MgCl_2$, 2.0 μmoles of ATP, 1.0 μmole of GTP, 20 μmoles of PEP, 50 μg of pyruvate kinase, 5 μmoles of mercaptoethanol, 1.0 μc of L-[^{14}C]leucine, and 12.0 mg of supernatant proteins. The final volume was 1.55 ml.

The polyribosomal pellets were gently resuspended in solution B and the suspension was centrifuged at 10,000g for 5 min to remove large particles. Aliquots of the supernatant material corresponding to 0.6 g of thyroid were added to incubation mixtures containing 1.0 μmole of ATP, 10 μmoles of PEP, 0.4 μmole of GTP, 30 μmoles of Tris-HCl (pH 7.6), 2 μmoles of mercaptoethanol, 50 μg of pyruvate kinase, 0.5 μc of L-[^{14}C]leucine, and supernatant fraction from 0.06 g of thyroid. The final volume was 1.0 ml.

The incubation was carried out by shaking in a 37° water bath. Following incubation, the reaction was stopped by the addition of twice the volume of 5% TCA and the precipitated protein was treated by the method of Siekevitz (1952). The precipitate was dissolved in 1.0 ml of formic acid and an aliquot of 0.4 ml was added to 4.0 ml of absolute alcohol and 10 ml of a scintillation fluid. The latter contained 0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis[2-(5-phenyloxazolyl)]-benzene in toluene. Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer at 40% efficiency.

Preparation of Thyroid-Soluble Protein for Sucrose Gradient Centrifugation. Following incubation, the volume of the mixture containing microsomes or ribosomes was brought to 2.0 ml with twice-distilled water and then to 5.0 ml with solution C or D, respectively. The resulting mixture was centrifuged at 140,000g for 1 hr. The protein in the supernatant fluid was precipitated by the addition of saturated ammonium sulfate (pH 6.8) and treated as previously described (Seed and Goldberg, 1965a; Sellin and Goldberg, 1965).

Sucrose Gradient Fractionation. Linear gradients were made with 5–20% sucrose in PBS. The SW 25.1 rotor was centrifuged in the Spinco L2-50 ultracentrifuge at 23,000 rpm for 40 hr at 3°. The optical density at 280 mμ was recorded automatically in the Gilford absorbance recorder. Fractions of 10 drops were collected in all experiments and an aliquot was added to 10 ml of Bray's (1960) solution. The samples were counted in a Packard Tri-Carb liquid scintillation spectrometer at 50% efficiency.

In some experiments, the fractions corresponding to 19S and 3–8S proteins, respectively, on the sucrose gradient were pooled and the proteins were precipi-

[1] Abbreviations used: PBS, phosphate-buffered saline (0.15 M sodium chloride in 0.01 M potassium phosphate (pH 6.8)); solution A, 0.25 M sucrose, 0.025 M KCl, 0.005 M $MgCl_2$, and 0.035 M Tris-HCl (pH 7.8); solution B, 0.06 M Tris-HCl (pH 7.6), 0.004 M mercaptoethanol and 0.005 M $MgCl_2$; solution C, 0.25 M sucrose, 0.025 M KCl, 0.005 M $MgCl_2$, and 0.05 M Tris-HCl (pH 7.6); solution D, 0.39 M sucrose, 0.025 M KCl, 0.005 M $MgCl_2$, and 0.05 M Tris-HCl (pH 7.6); DOC, sodium deoxycholate; TCA, trichloracetic acid; PEP, phosphoenolpyruvate; GTP, sodium salt of guanosine triphosphoric acid; ATP, sodium salt of adenosine triphosphoric acid.

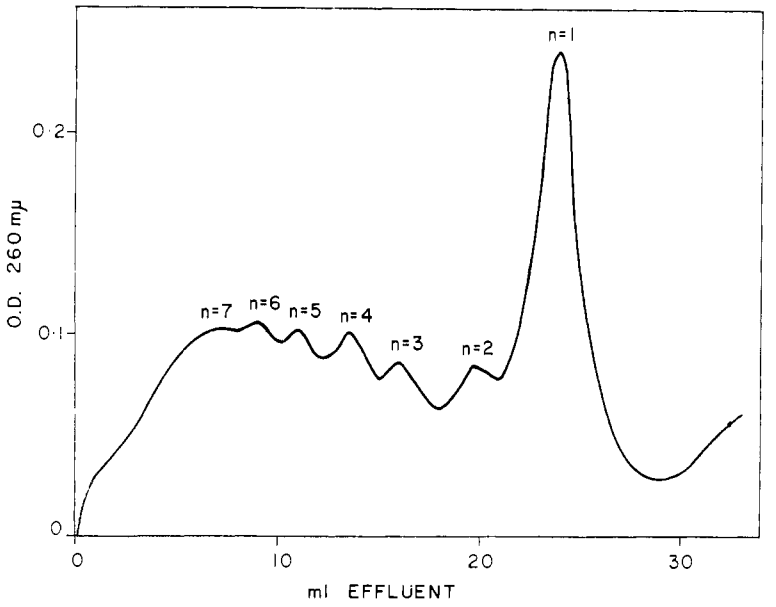

FIGURE 1: Sucrose density gradient profile of thyroid ribosomes isolated from the postmitochondrial supernatant fraction. Number of ribosomes per aggregate = *n*. A ribosomal pellet suspension (1.0 ml) was layered onto linear 10–30% sucrose gradient (29-ml total). The gradient solution also contained 0.003 M $MgCl_2$, 0.025 M KCl, and 0.05 M Tris-HCl (pH 7.6). The chamber temperature was maintained at −5° and the gradient was centrifuged in a Spinco SW 25.1 rotor at 22,500 rpm for 5 hr. The optical density at 260 mμ was recorded automatically in the Gilford absorbance recorder.

tated with 50% ammonium sulfate. The precipitate was then dissolved in a minimum of PBS and recentrifuged on a similar sucrose gradient.

Antibody Precipitation of Sucrose Gradient Fractions. Antibody precipitation of the protein of sucrose gradient fractions was carried out as previously described (Sellin and Goldberg, 1965), except that the nonspecific system consisted of 0.01 ml of antiserum to DNP-bovine plasma albumin in the presence of 75 μg of DNP-bovine plasma albumin. In addition, precipitation of the soluble complex formed between rabbit antithyroglobulin antibody and labeled 3–8S protein was accomplished using antirabbit γ-globulin antibody.

Protein and RNA Determinations. Protein contents of the microsomal and supernatant fractions were determined by the biuret method (Layne, 1962) and those of the ribosomal fraction by the method of Lowry *et al.* (1951). The RNA content of the ribosomal fraction was estimated from the absorbancy at 260 mμ using the relation: 20 absorbancy units = 1.0 mg of RNA (Wettstein *et al.*, 1963).

Results

Amino Acid Incorporation into Proteins by the Microsomal Fraction of Calf Thyroid Gland. As has been shown for sheep thyroid gland (Singh *et al.*, 1965; Nunez *et al.*, 1965b; Soffer, 1966), a calf thyroid microsomal fraction incorporates [^{14}C]leucine into polypeptide (Table I). The incorporation requires ATP and an energy-generating system and is inhibited 80% by 5×10^{-4} M puromycin. Further, the incor-

TABLE I: Incorporation of L-[^{14}C]Leucine into Protein by Calf Thyroid Microsomal Fractions.

Experimental Conditions	Sp Act. (cpm/mg of protein)
Expt 1	
Complete[a]	389
Minus ATP, PEP, pyruvate kinase	3
Expt 2	
Complete	253
Minus particles	10
Puromycin 5×10^{-4} M	49
Expt 3	
Complete	524
Minus supernatant fraction	169

[a] The incubation mixture contained the following in a final volume of 1.55 ml: 28 μmoles of Tris-HCl buffer (pH 7.8), 200 μmoles of sucrose, 70 μmoles of KCl, 15 μmoles of $MgCl_2$, 2.0 μmoles of ATP, 1.0 μmole of GTP, 20 μmoles of PEP, 50 μg of pyruvate kinase, 5 μmoles of mercaptoethanol, 0.5 μc of L-[^{14}C]leucine (200 μc/μmole), 9.0 mg of particulate protein, and 12.0 mg of supernatant protein. Incubation was for 30 min.

TABLE II: Chemical Composition and Amino Acid Incorporating Activity of Calf Thyroid Polyribosomal Fractions.

	μg of RNA/ g of Thyroid	μg of Protein/ g of Thyroid	Ratio RNA: Protein	Sp Act. (cpm/mg of RNA)[a]	Sp Act. (cpm/mg of protein)
Av of 4 experiments	135	229	0.6	27,041	21,808

[a] The incubation mixture contains 1.0 μmole of ATP, 10 μmoles of PEP, 0.4 μmole of GTP, 7 μmoles of $MgCl_2$, 30 μmoles of Tris-HCl (pH 7.6), 50 μg of pyruvate kinase, supernatant fraction from 0.06 g of thyroid, 0.5 μc of L-[^{14}C]leucine (200 μc/μmoles), and polyribosomes from 0.6 g of thyroid. The final volume was 1.0 ml. Incubation was carried out at 37° for 45 min.

poration is depressed by 95 and 70%, respectively, when the particulate or supernatant proteins are omitted. The supernatant fraction can be replaced with equal efficiency by a mixture of amino acids (1×10^{-3} M) and the pH 5 enzyme was prepared according to Decken and Campbell (1962).

In order to determine the proportion of incorporated [^{14}C]leucine attached to the NH_2-terminal end of polypeptide chains, the labeled protein was treated with fluorodinitrobenzene and then hydrolyzed with HCl (Sanger, 1952). Since 95% of the radioactivity remained water soluble, the incorporated [^{14}C]leucine was almost entirely in internal peptide linkage or C terminal.

Polyribosomes: Isolation, Chemical Composition, and Amino Acid Incorporation. Sucrose density gradient analysis of ribosomal material prepared from the postmitochondrial supernatant fraction is shown in Figure 1. Seven distinct ribosomal peaks are obtained. About 25% of the material corresponds to single ribosomes while polyribosomal aggregates, greater than seven units (Wettstein *et al.*, 1963; Warner *et al.*, 1963), are also found. Further, a noticeable pellet containing RNA is always found at the bottom of the tube. The polyribosomal pattern is similar to that obtained with other tissues (Spector and Travis, 1966; Howell *et al.*, 1964; Munro *et al.*, 1964).

The results of analytical tests (summarized in Table II) show that 135 and 229 μg of RNA and protein, respectively, are obtained/g of wet weight of thyroid with a RNA protein ratio of 0.6. Under similar analytical conditions, however, the yield of RNA and protein is greater in rat liver and the ratio is nearly doubled (Wettstein *et al.*, 1963). The specific activity expressed as counts per minute per milligram of protein is more than 40-fold greater than that of the microsomal fraction (see Table I).

The optimal conditions for the incorporation of amino acids in the thyroid preparation are very similar to those of liver and lens (Wettstein *et al.*, 1963; Spector and Travis, 1966). Upon removal of ATP and an energy-generating system (Table III), virtually no incorporation is observed. Without the polyribosomal fraction, supernatant proteins, or Mg^{2+} incorporation of [^{14}C]leucine is inhibited by about 95%, while the omission of GTP diminished the incorporation by about 60%.

TABLE III: Incorporation of L-[^{14}C]Leucine into Protein by Calf Thyroid Polyribosomal Fraction.

Incubation Mixture	Cpm of L-[^{14}C]-Leucine Incorp
Complete[a]	1060
Minus ATP, PEP, pyruvate kinase	12
Minus GTP	416
Minus supernatant fraction	55
Minus ribosomes	36
Minus $MgCl_2$	46

[a] Incorporation assay is described in Table II.

Time Course of Incorporation of Amino Acid into Proteins. The incorporation of [^{14}C]leucine into total protein by both microsomal and ribosomal fraction reaches a maximum at about 45 min and then levels off. During incubation, labeled proteins appear in the supernatant fluid (Figure 2). The incorporation of leucine into the particulate fraction is rapid, reaches a maximum at about 15 min, and then declines, while a slow incorporation into the soluble fraction continues for at least 180 min. At this time, the labeled soluble proteins account for about 25% of the total radioactivity incorporated. A marked difference is observed with the ribosomal fraction where 45% of the labeled protein is already soluble after 1 hr of incubation and continues to increase. These findings are similar to those reported by Allen and Schweet (1962) with reticulocyte ribosomal preparations.

Sucrose Density Gradient Centrifugation Analysis of Labeled Particulate and Soluble Proteins. It has been shown with lamb thyroid slices (Sellin and Goldberg, 1965) that the labeled proteins solubilized from subcellular particles sediment in the 19S and 3–8S regions of the sucrose gradient (a 12S fraction may be more readily extracted and appears in the particle wash). Similar attempts failed to demonstrate two well-defined populations of proteins when the *microsomal* proteins, labeled *in vitro*, are extracted with digitonin. About 20% of the radioactivity is solubilized and is found in a very slowly sedimenting fraction (3–8 S) with no significant label in the thyroglobulin region (Figure

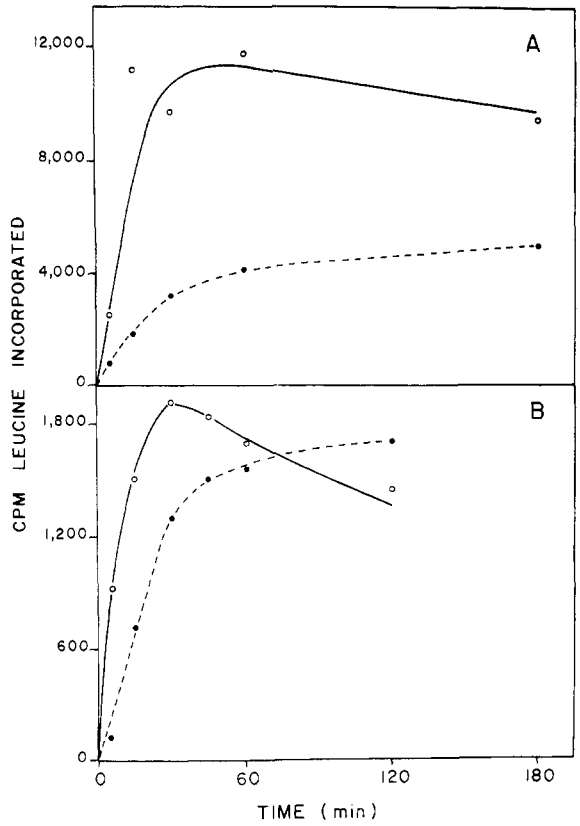

FIGURE 2: Time course of incorporation of L-[^{14}C]-leucine into protein by microsomal and ribosomal fractions of calf thyroid. (A) Microsomal fraction. The reaction mixture and conditions of incubation were the same as those described in Table I, except that 10 μc of L-[^{14}C]leucine was used. (B) Ribosomal fraction. The reaction mixture and conditions of incubation were the same as those described in Table II, except that 1 μc of L-[^{14}C]leucine was used. (O——O) Counts per minute of leucine incorporated into particulate proteins; (●- - - -●) counts per minute of leucine incorporated into soluble proteins.

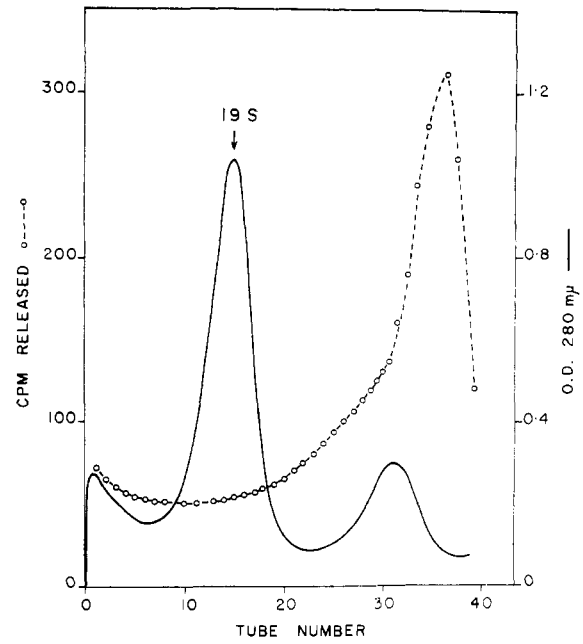

FIGURE 3: Sucrose density gradient centrifugation analysis of label extracted from particulate proteins. The experimental conditions were the same as those described in Table 1 and Figure 2. At the end of 2-hr incubation, the mixture was treated as described in the Experimental Section. The microsomal pellet was resuspended by gentle homogenization in 5.0 ml of solution A and recentrifuged at the same speed for the same time. The microsomal pellet was washed twice and then extracted with 0.5% digitonin. Unlabeled supernatant protein was added before sucrose density gradient centrifugation. The position on this gradient of the 19S thyroglobulin optical density peak is indicated by the arrow.

3). With 0.5% deoxycholate, 40% of the labeled proteins is extracted but still sediments mainly in the 3–8S region. Similar results are obtained with the labeled proteins released from the thyroid ribosomal pellet after incubation with ribonuclease (Baglioni and Colombo 1964). However, centrifugation analysis of the labeled *soluble* proteins derived from incubations containing microsomes (Figure 4A) shows that more than 30% of the label is in the thyroglobulin region of the gradient and has a peak sedimentation constant of about 18 S, similar, therefore, to the material labeled in thyroid slices (Seed and Goldberg, 1965a). The remaining radioactive protein sediments more slowly in a broad peak from 3 to 12 S. Using sheep thyroid microsomal fractions, Nunez *et al.* (1965a) have recently reported similar observations. By contrast, the pattern produced by centrifugation analysis of the labeled soluble proteins from the ribosomal fraction (Figure 4B) is similar to those obtained with labeled proteins extracted from the microsomal and ribosomal pellets (see Figure 3). Almost all the radioactivity is present in the 3–12S region of the gradient. Less than 10% of the label is generally found in the 18S thyroglobulin region. Soffer (1966) and Cartouzou *et al.* (1967) have recently also found ribosomes incubated *in vitro* to be inadequate in producing thyroglobulin.

Antibody Precipitation of Labeled Soluble Thyroid Proteins. In Figure 5 the ability of antithyroglobulin antibody to precipitate labeled soluble thyroglobulin-like protein obtained upon incubation of thyroid slices or the microsomal fraction was compared. It can be seen that there is a specific precipitation of the labeled 18S protein in both cases suggesting a close similarity in structure and chemical composition between the labeled protein synthesized *in vitro* and native 19S thyroglobulin. Similar attempts with the thyroglobulin-like protein labeled with [^{14}C]leucine in the ribosomal system have not been uniformly successfull In some experiments, however, a portion of the smal.

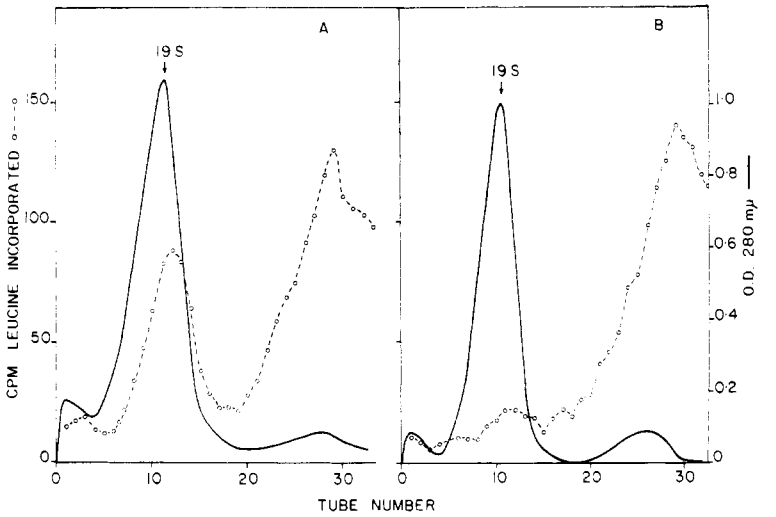

FIGURE 4: Sucrose density gradient centrifugation analysis of labeled soluble protein. The experimental conditions were the same as those described in Tables I and II and Figure 2. At the end of 2-hr incubation, the labeled soluble protein was prepared as described in the Experimental Section. (A) From incubation with microsomes. (B) From incubation with ribosomes. The position on this gradient of the 19S thyroglobulin optical density peak is indicated by the arrow.

amount of radioactivity associated with the thyroglobulin peak appeared to be specifically precipitated with antithyroglobulin antibodies.

As in experiments using thyroid slices (Sellin and Goldberg, 1965), no significant radioactivity associated with the 3–8S proteins was specifically precipitated by antithyroglobulin antibody. It could be shown, however, that this antibody does form a nonprecipitating complex with a fraction of these labeled proteins which can be precipitated by antibodies against rabbit γ-globulin. In fact, at different positions on a sucrose gradient from 10 to 25% of the labeled protein was precipitated (Table IV), suggesting that, at most, only a small portion of the radioactive 3–8S protein was related to thyroglobulin.

TABLE IV: Antibody Precipitation of 3–8S Proteins.[a]

	Tube 24 (cpm)	Tube 29 (cpm)
Anti-DNP-BPA precipitate	9	14
Anti-TG precipitate	7	8
Anti-γ-globulin precipitate	33	75
Original	149	678

[a] The labeled 3–8S proteins were purified by sucrose density gradient centrifugation. Two individual fractions on the sucrose gradient were precipitated with antihydroglobulin antiserum (anti-TG precipitate) after preliminary treatment with antiserum to DNP-bovine plasma albumin (anti-DNP-BPA precipitate). Then, the supernatant was treated with antirabbit γ-globulin antiserum (anti-γ-globulin precipitate) and the precipitated radioactive protein was determined. Original represents the total radioactivity initially present in each tube.

Discussion

The present studies describe microsomal and ribosomal systems from calf thyroid glands which incorporate L-[^{14}C]leucine into both particle-bound and soluble proteins. In both cases, the requirements for incorporation of amino acid into peptide linkage are those standard for cell-free protein synthesis by mammalian systems. As might be expected, the specific activity of the ribosomal fraction, expressed as counts per minute per milligram of particulate proteins, is much greater than that of the microsomal fraction. The yield of rRNA from 1.0 g wet wt of thyroid gland is about 10% of that obtained with rat liver under similar experimental conditions (Wettstein *et al.*, 1963). This difference can be attributed to the smaller number of epithelial cells per gram of thyroid compared with liver. The RNA is found under seven discrete polyribosomal peaks and as much as 25% of the optical density is associated with ribosomal aggregates containing more than seven units.

The release of labeled proteins into the supernatant fluid during incubation is a slow process and accounts for less than 25% of the total radioactivity incorporated by the thyroid microsomal fraction. Comparable data have been found with rat liver microsomes (Korner, 1961), and Redman *et al.* (1966) recently reported a similar observation with pigeon pancreas microsomes. The latter authors suggested that the soluble proteins might result from the relatively large

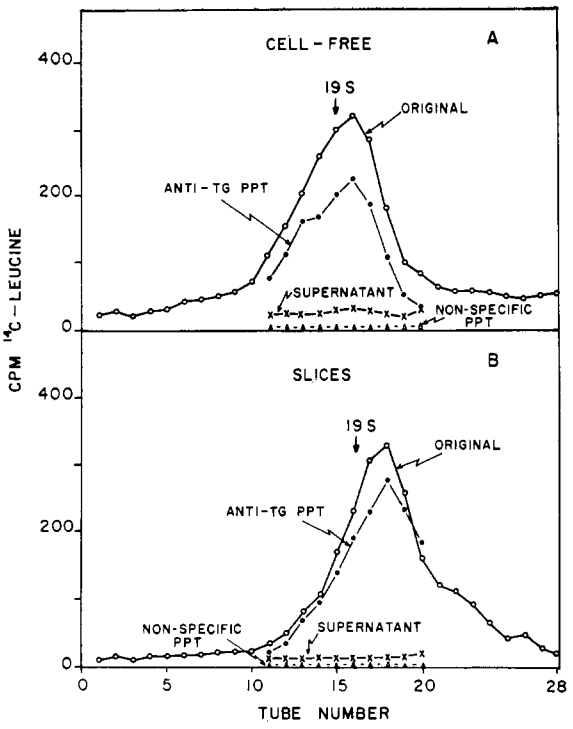

FIGURE 5: Antibody precipitation of labeled soluble thyroid proteins obtained from slices and microsomal fraction. Thyroid slices, prepared as previously described (Seed and Goldberg, 1965a), or the microsomal fraction were incubated 2 hr at 37°. The labeled thyroglobulin-like protein was purified by sucrose density gradient. Individual gradient fractions were precipitated with antithyroglobulin antiserum (anti-TG precipitate) after preliminary treatment with antiserum to DNP-bovine plasma albumin (nonspecific precipitate) as described in the Experimental Section. *Original* represents the total radioactivity initially present in each tube. The position on this gradient of the 19S thyroglobulin optical density peak is indicated by the arrow. (A) Cell-free system. (B) Slices.

(25%) percentage of ruptured microsomal vesicles at the end of the incubation time and/or by diffusion of the proteins from the vesicles into the medium. Previous experiments from their laboratory had shown that proteins newly synthesized *in vivo* are released across the membrane of the endoplasmic reticulum into the cisternal space (Palade *et al.*, 1962; Caro and Palade, 1964). Further, their results indicate that the pigeon pancreas microsomal fraction *in vitro* retains the mechanism necessary for the transfer and release of protein into the vesicles, since more than 50% of the incorporated radioactivity is released upon treatment with deoxycholate. Digitonin or deoxycholate extraction of the thyroid microsomal fraction solubilizes 20 or 40%, respectively, of the radioactivity, suggesting that a similar mechanism for release of newly synthesized and presumably finished proteins into the cisternal space is present in the calf thyroid microsomal system.

On the other hand, 45% of the labeled proteins is already soluble after 1-hr incubation with the thyroid ribosomal system, and this continues to increase slowly over a prolonged period of time. This suggests that the mechanism involved in the release of proteins from their ribosomal site of synthesis is present in the ribosomal complex. It is not excluded, however, that the label released into the medium arises as a result of breakdown of the ribosomes during incubation.

The present results do not indicate whether the isolated microsomal system is capable of initiating the synthesis of new molecules, or only of completing those molecules whose synthesis has been started *in vivo*. Further, we do not know whether the protein molecules synthesized on the ribosomes and released into the cisternal space are subunits of thyroglobulin which are to undergo some transformation (carbohydrate moiety attachment, S–S bond formation, etc.) (Robinson *et al.*, 1964; Molnar *et al.*, 1964; Sarcione *et al.*, 1964; Sinohara and Sky-Peck, 1965; Helgeland, 1965; Goldberger *et al.*, 1964; Venetioner and Straub, 1965; DeLorenzo *et al.*, 1966) before aggregation to form thyroglobulin. Labeled proteins extracted from the *microsomal pellets* after a 2-hr incubation are found in a very slowly sedimenting fraction (3–8 S) with no significant radioactivity in the thyroglobulin region of the gradient. These data differ from those obtained with thyroid slices where the label extracted from particulate fractions is found in both the 3–8S and 18S proteins (Sellin and Goldberg, 1965). However, an appreciable amount of the labeled *soluble proteins* synthesized in the microsomal system sediments at 17–18 S, and is specifically precipitated with antithyroglobulin antibodies, similar, therefore, to the immature form of thyroglobulin (Seed and Goldberg, 1965a,b; Goldberg and Seed, 1965; Nunez *et al.*, 1965b) obtained with thyroid slices. It is possible that the thyroglobulin-like material is released into the medium as rapidly as it is formed during incubation of the microsomal fraction, thus accounting for its absence from the microsomal extracts. This may be a consequence of the *in vitro* incubation conditions. It has been recently found that the proportion of newly made γ-globulin associated with the microsomes of lymph node cells depends on the composition of the medium used for isolation of the particles (Swenson and Kern, 1967). Since the morphological and functional properties of isolated thyroid microsomal fraction have not been characterized, it is also possible that labeled 18S protein prepared from microsomes labeled in slices is associated with structures (*e.g.*, apical vesicles) not directly concerned with polypeptide formation. It would not be expected that such structures would be labeled in the cell-free system.

In vitro incorporation experiments with [^{14}C]-glucose have shown that thyroid microsomal particulate proteins are labeled (Spiro and Spiro, 1966), while ribosomal proteins are not (Cartouzou *et al.*, 1967). Similar data have been reported from experiments *in vivo* and with slices (Sarcione, 1964; Bouchilloux and Cheftel, 1966; McGuire *et al.*, 1965) and suggest

that the membrane-containing systems attach carbohydrate to protein synthesized by the ribosomes. Similarly, the finding of labeled thyroglobulin in the soluble fraction of incubated microsomes but not ribosomes indicates a possible role for the membrane structures in subunit aggregation. On the other hand, the inability of the ribosomal fractions employed here to synthesize significant amounts of thyroglobulin-like protein may be due to the fact that the ribosomal aggregates which are composed of mainly seven and fewer units are too small to synthesize complete peptide chain subunits (mol wt 150,000; de Chrombrugghe *et al.*, 1966) which can aggregate to larger forms. The fact that some labeled proteins obtained on incubation of ribosomes have a sedimentation constant of 17–18 S and are precipitable by antithyroglobulin antibodies, may reflect complete chain synthesis by a small fraction of the ribosomes, presumably the very large aggregates. Finally, it should be pointed out that these studies were not designed to demonstrate net synthesis of thyroglobulin-like molecules, so that labeling of the 18S protein by an exchange of subunits (see Goldberg and Seed, 1965) has not been eliminated.

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