Effect of Thyroid Hormones on the Level of the Hepatic mRNA for α_{2u} Globulin†

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ABSTRACT: A procedure is presented for the purification of α_{2u} globulin, a protein synthesized in the liver, secreted into the serum, and excreted in the urine of male rats. The steady-state levels of α_{2u} globulin in the serum and liver cytosol fraction of adult male rats have been determined using a radial immunodiffusion assay. A cell-free protein synthesizing system, easily prepared from commercial wheat germ, has been used to identify and quantitate the mRNA coding for α_{2u} globulin. Poly(A)-containing RNA isolated from male rat liver directs the synthesis in the wheat germ translational system of a protein which is precipitated by rabbit anti-α_{2u} globulin and migrates with authentic α_{2u} globulin on sodium dodecyl sulfate-polyacrylamide gels. Poly(A)-containing RNA from the livers of female rats or from the kidneys of male rats, tissues which synthesize no α_{2u} globulin, do not direct the synthesis of α_{2u} globulin in the wheat germ system. Thyroidectomized male rats had no detectable α_{2u} globulin in their sera or liver cytosols, and the livers from these thyroidectomized males were found to contain no translatable mRNA for α_{2u} globulin, as measured in the wheat germ system. Administration of L-thyroxine or triiodo-L-thyronine to thyroidectomized males resulted in the synthesis of α_{2u} globulin, as measured by increased levels of this protein in sera and liver cytosols. This increase in α_{2u} globulin synthesis following thyroid hormone treatment was accompanied by a parallel increase in the functional level of the hepatic mRNA coding for this protein. Treatment of thyroidectomized males with a variety of androgens failed to stimulate α_{2u} globulin synthesis, and no α_{2u} globulin mRNA could be detected in the livers from these androgen-treated thyroidectomized males. These findings indicate that thyroid hormones influence α_{2u} globulin synthesis in male rat liver by acting pretranslationally, possibly by modulating gene transcription, and rule out the possibility of an indirect androgen-mediated effect of thyroid hormones in modulating α_{2u} globulin biosynthesis.

Alpha$_{2u}$ globulin is a protein found by Roy and Neuhaus (1966a) to be in the urine of mature male rats and absent from the urine of female rats. Its function is, thus far, unknown. Liver perfusion and immunofluorescent studies have shown that α_{2u} globulin is synthesized in the liver of the male rat, secreted into the serum, and excreted in the urine (Roy and Neuhaus, 1966b; Roy and Raber, 1972). The hepatic synthesis of α_{2u} globulin is under complex hormonal control: androgens, glucocorticoids, growth hormone, and thyroid hormones act as inducers of α_{2u} globulin, while estrogens have been shown to repress its synthesis (Roy and Neuhaus, 1967; Irwin et al., 1971; Roy, 1973; Roy and Leonard, 1973).

Reports from various laboratories have shown that hormonal regulation of the synthesis of specific proteins can occur via modulation of the level of the specific mRNA coding for these proteins (Rhoads et al., 1973; Chan et al., 1973; Palmiter and Smith, 1973; Schutz et al., 1973). An earlier report from this laboratory (Sippel et al., 1975) indicated that the regulation of the synthesis of α_{2u} globulin by glucocorticoids occurs through modulation of the level of the hepatic mRNA coding for α_{2u} globulin.

It was reported by Roy (1973) that thyroidectomy of mature male rats depresses the urinary output of α_{2u} globulin. Administration of L-thyroxine restores the urinary excretion of this protein to normal levels after 8–10 days of hormone administration. There have been recent reports (Surks et al., 1973; Oppenheimer et al., 1974) concerning the existence of specific hepatic nuclear binding sites for thyroid hormones. In contrast to earlier suggestions (Litwack, 1964) which postulated direct thyroid hormone effects on cytoplasmic organelles, these thyroid hormone receptor studies, in conjunction with the reports of increased RNA synthesis due to thyroid hormones (Tata, 1966; Griswold and Cohen, 1973), indicate the possibility of direct effects of thyroid hormones upon transcription in their target tissues.

This paper describes a simple procedure for the purification of α_{2u} globulin from male rat urine, the preparation of rabbit anti-α_{2u} globulin, and the use of a wheat germ cell-free translational system to quantitate the level of the hepatic mRNA coding for α_{2u} globulin. Studies are herein reported exploring the effects of thyroid hormones on the hepatic level of the specific mRNA for α_{2u} globulin, a protein which has been shown to be thyroid hormone inducible.

Materials and Methods

Purification of α_{2u} Globulin. Because we were unable to prepare reasonable amounts of pure α_{2u} globulin from urine using the published purification procedures (Roy and Neuhaus, 1966b; Royce, 1968), a new purification scheme was devised. Urine was collected from adult (>80 days old) male rats kept in stainless steel metabolism cages. The urine was frozen and kept at −20 °C until use. The urine (300–500 ml) was filtered through a Whatman No. 1 filter paper, and a 50–75% $(NH_4)_2SO_4$ precipitate was prepared. The precipitate was dissolved in 25 ml of 0.05 M NH_4OAc (pH 5.0) and dialyzed overnight at 4 °C against the same buffer. The dialysate was applied to a 2.5 × 25 cm carboxy-

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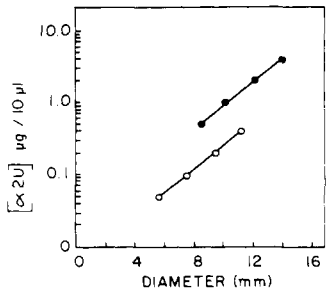

FIGURE 1: Radial immunodiffusion assay for α_{2u} globulin. Diameters of precipitin rings were measured after 12 h at room temperature. Plates contained: 12.5 mg of anti-α_{2u} IgG (●) or 5.0 mg of anti-α_{2u} IgG (○) in 15 ml of 1% buffered agar.

methylcellulose column (CM-52, Whatman Co.) previously equilibrated with 0.05 M NH_4OAc (pH 5.0). After application of the sample, the column was washed with 300 ml of the equilibration buffer, followed by 250 ml of 0.2 M NH_4OAc (pH 5.0). The major protein peak eluting with the 0.2 M buffer was collected, dialyzed against distilled water, and lyophilized. The lyophilized material was dissolved in 10–12 ml of PBS[1] and applied to a Sephadex G-75 column (2.5 × 40 cm) previously equilibrated with PBS. Elution was continued with PBS, and the major protein peak was collected and judged to be homogeneous by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue. This procedure typically resulted in yields of homogeneous α_{2u} globulin ranging from 45 to 60% of urinary α_{2u} globulin (Table I).

Preparation of Antiserum. α_{2u} globulin, purified to homogeneity by the method described above, was used to prepare monospecific rabbit anti-α_{2u} globulin IgG. Intracutaneous injections of an emulsion containing 200 μg of α_{2u} globulin in 1 ml of PBS plus 1 ml of Freunds Complete Adjuvant were made weekly for 4 weeks. The rabbits were bled on the fifth week. An additional 200 μg of α_{2u} globulin was injected on the sixth week, and the rabbits were rebled the following week. The rabbit IgG serum fraction precipitating between 0 and 40% saturation with respect to $(NH_4)_2SO_4$ was used as antiserum. The determination of the antigen–antibody equivalence was done as described previously (Sippel et al., 1975).

Immunoassay of α_{2u} Globulin. α_{2u} globulin was assayed using a radial immunodiffusion technique as described by Irwin et al. (1971), with modifications. A solution of anti-α_{2u}, 5.0 to 12.5 mg of protein in 7.5 ml of a buffer containing 0.03 M potassium phosphate (pH 8.0) and 0.1 M NaCl, was mixed with 7.5 ml of 2% agar in the same buffer at 50–54 °C. After mixing, the solution was quickly poured into a plastic petri dish, 8.5 cm in diameter. The agar was allowed to harden, and 2-mm diameter wells were cut (12–14 per plate) with a tubular cutter. Ten microliters of known concentrations of purified α_{2u} globulin was placed in 4 of the wells, and 10 μl of the unknown samples was placed in the remaining wells. The plates were left at room temperature for 12–14 h. The diameters of the resultant precipitin rings around the wells were measured with a ruler and found to be proportional to log [α_{2u}] (Figure 1). By varying the amount of anti-α_{2u} IgG in the agar, different ranges of α_{2u} globulin concentrations could be measured accurately. Using this technique, determinations of α_{2u} globulin concentrations could be made over a range of 5.0–600 μg/ml.

Table I: α_{2u} Globulin Purification.

Fraction	[Protein] (mg/ml)	[α_{2u}] (mg/ml)	mg α_{2u}/ mg Protein	mg α_{2u}	% Yield
Urine	3.2	0.61	0.19	153	100
50–75% $(NH_4)_2SO_4$	9.7	4.8	0.49	115	75.0
CM-cellulose	0.76	0.73	0.96	91	59.5
Sephadex G-75	0.82	0.82	1.00	74	48.5

Animals. Surgically thyroidectomized male rats (7 weeks old) were purchased from Carworth Ltd., Boston, Mass., and maintained on 1% $CaCl_2$ in their drinking water throughout the course of the experiment, in a room with a controlled light cycle. To ensure total thyroidectomy, the rats were kept on a low iodine diet (Nutritional Biochemicals Co.) for 10 days and then given an intraperitoneal injection of $Na^{131}I$, 100 μCi/rat. One month later, the rats were judged thyroidectomized by growth parameters.

Hormone Administration. Triiodo-L-thyronine (T3) was injected intraperitoneally (100 μg/100 g body weight) in PBS with 2% bovine serum albumin. L-Thyroxine (T4) was injected intraperitoneally (10 μg/100 g) in 5 mM NaOH. Where indicated, androgens were injected subcutaneously (200 μg/100 g) in an emulsion containing (by volume) 89.6% 0.1 M sodium phosphate (pH 7.2), 10% propylene glycol, and 0.04% Tween 80.

Preparation of Rat Liver Cytosol (S100). Rats were sacrificed by cervical dislocation, and the livers were removed, minced with scissors, and homogenized in 3 vol of ice-cold 0.25 M sucrose, 50 mM Tris (pH 7.5), 25 mM NaCl, and 5 mM $Mg(OAc)_2$ using 8 strokes with a Potter-Elvehjem homogenizer with a loose-fitting Teflon pestle. To this homogenate was added a 1/9 volume of 10% Triton X-100 and 10% deoxycholate and the mixture was rehomogenized using 8 strokes with a tight-fitting Teflon pestle. This homogenate was centrifuged for 10 min at 21 000*g* at 2 °C. The upper fatty layer of the supernatant was removed by aspiration, and the supernatant, containing the hepatic α_{2u} globulin, was centrifuged for 2 h at 110 000*g* to pellet the polysomes. The supernatant (S100) was defatted as above and its α_{2u} globulin content determined using the radial immunodiffusion assay.

Preparation of Rat Liver mRNA. RNA was prepared from 2.5 g of frozen rat liver as described previously (Schutz et al., 1973). Total hepatic RNA extracted with phenol–chloroform was chromatographed on cellulose (Sigmacell 38) to isolate the poly(A)-containing fraction (Schutz et al., 1972). The yield of poly(A)-containing RNA varied from 0.15 to 0.24 mg/g of liver.

Preparation of Wheat Germ Translational System (WG S30). Raw wheat germ (Old Stone Mill) was purchased, vacuum sealed, from Niblack Foods, Rochester, N.Y. Highly active protein synthesizing systems could be prepared only from raw wheat germ kept in its original vacuum until use. A cell-free protein synthesizing system was prepared following the procedure of Marcu and Dudock (1974), with modifications: 20 g of raw wheat germ was ground in an ice-cooled mortar with 20 g of crushed glass

[1] Abbreviations used are: PBS, 0.01 M sodium phosphate (pH 7.2)–0.14 M NaCl; IgG, immunoglobulin G; T3, triiodo-L-thyronine; T4, L-thyroxine; H, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

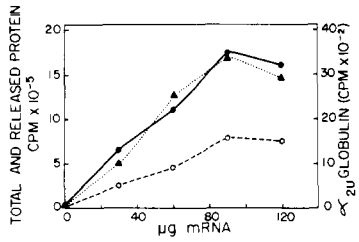

FIGURE 2: Dependence of in vitro protein synthesis on exogenous mRNA. [^{3}H]Leucine incorporation into total (●) and released chain (○) Cl_3CCOOH-precipitable counts after 60-min incubation at 30 °C. Incorporation of [^{3}H]leucine into α_{2u} globulin (▲) immunoprecipated from 400 μl of the released polypeptide chain fraction.

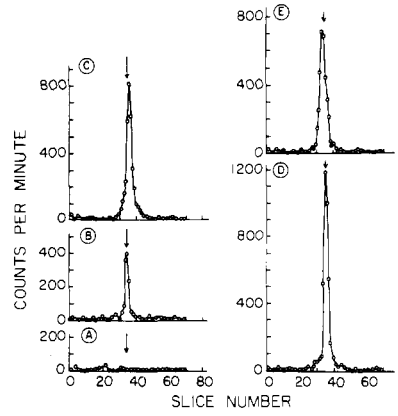

FIGURE 3: Sodium dodecyl sulfate-polyacrylamide gel electrophoretic profiles of immunoprecipitated α_{2u} globulin synthesized in vitro. Protein synthesis performed as described under Materials and Methods. α_{2u} globulin immunoprecipitated from 400 μl of released polypeptide chain fraction of the WG S30 system containing: (A) 0, (B) 30, (C) 60, (D) 90, and (E) 120 μg of male rat liver mRNA. Arrows mark position of authentic α_{2u} globulin.

for 1–1.5 min. To this paste was added 40 ml of a buffer containing 20 mM Hepes (pH 7.6), 100 mM KCl, 1 mM $Mg(OAc)_2$, 2 mM $CaCl_2$, and 1 mM dithiothreitol, and the mixture was swirled slowly for 1 min. The thick slurry was scraped into tubes and centrifuged at 30 000*g* for 12 min at 2 °C. The upper fatty layer of the resultant supernatant was carefully removed by aspiration, and the total supernatant was collected and applied to a Sephadex G-25 Fine column at 4 °C previously equilibrated with 20 mM Hepes (pH 7.6), 120 mM KCl, 5 mM $Mg(OAc)_2$, and 1 mM dithiothreitol. After application of the sample, the column was eluted with this buffer, and the void volume fractions (2 ml/fraction) with A_{260} values of >90 units/ml were collected and pooled. The combined eluate was centrifuged at 30 000*g* for 20 min at 2 °C. The supernatant (generally 20–25 ml) was found to have an A_{260} of 120–135 units/ml and an A_{280} of 80–90 units/ml. This supernatant (WG S30) was quick frozen by dropwise addition directly into liquid nitrogen using a Pasteur pipet. The frozen beads were collected and stored at 90 °C.

In Vitro Protein Synthesis. For in vitro protein synthesis, the complete reaction mixture, in a 500-μl volume, contained: 20–25 A_{260} units (150–200 μl) of WG S30, 20 mM Hepes (pH 7.6), 2 mM dithiothreitol, 2.5 M $Mg(OAc)_2$, 100 mM K^+, 110 μCi of [^{3}H]leucine (50 Ci/mmol), 32 μM of each of the other 19 natural amino acids, 0.8 mM ATP, 0.08 mM GTP, 0.5 mM CTP, 4.0 mM creatine phosphate, 0.06 mg of creatine phosphokinase, and 100 μg of rat liver mRNA. After incubation at 30 °C for 1 h, the amount of [^{3}H]leucine incorporated into total protein was determined in a 10-μl aliquot by the method described by Bollum (1968). Released polypeptide chains were separated from polysomes by centrifugation at 150 000*g* for 1 h at 2 °C. The amount of [^{3}H]leucine incorporated into released chains was similarly determined in a 10-μl aliquot.

Quantitation of α_{2u} Globulin Synthesized in Vitro. The amount of α_{2u} globulin synthesized in vitro was determined as described by Sippel et al. (1975): the released polypeptide chain fraction was brought to 2% Triton X-100–0.01 M leucine in PBS. Ten micrograms of unlabeled carrier α_{2u} globulin was added, followed by 150 μl of anti-α_{2u} solution (50 mg of IgG/ml). The solution was left at room temperature for 30 min and then placed at 4 °C for 2–4 h. The resultant immunoprecipitate suspension (1.5 ml) was layered over 2 ml of 1 M sucrose in 2% Triton X-100–0.01 M leucine in PBS. The immunoprecipitate was centrifuged through the sucrose layer at 2000*g* for 15 min. The top layer was removed by aspiration, and the surface of the sucrose layer was washed twice with 2 ml of PBS. The sucrose layer was then removed, and the pellet was washed 5 times with hard vortexing with 4 ml of PBS. These repeated washings of the pellet were found to be necessary to remove nonspecific labeled proteins contaminating the immunoprecipitate. The washed precipitate was then dissolved in 100 μl of 2% sodium dodecyl sulfate, 2% β-mercaptoethanol, 10 mM sodium phosphate (pH 7.2), and 10% glycerol, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Weber and Osborn, 1969) on 0.1% sodium dodecyl sulfate–10% polyacrylamide gels at 8 mA/gel for 4.5 h. After electrophoresis, the gels were frozen and cut into 1-mm slices, and the radioactivity of the slices was determined as described (Sippel, 1973) using Yorktown Hydromix. Protein markers, including pure α_{2u} globulin, were run on parallel gels and stained with Coomassie brilliant blue.

The in vivo rate of synthesis of α_{2u} globulin was determined using intravenous injections of [^{3}H]leucine, as described by Sippel et al.[2] Protein determinations were done using the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Results

mRNA Dependent Synthesis of α_{2u} Globulin and Total Protein in the WG S30 System. The extent of [^{3}H]leucine incorporation into total protein and into released polypeptide chains in the wheat germ translational system was a linear function of the amount of hepatic mRNA added (Figure 2). Saturation was not achieved until a relatively high amount (90–100 μg/500 μl) of mRNA was added, enabling a high level of [^{3}H]leucine incorporation into protein. After 1-h incubation with 100 μg of rat liver mRNA the incorporation of [^{3}H]leucine into total and released protein reached 206 and 94 pmol/500 μl, respectively.

The identification and quantitation of the mRNA-dependent synthesis of α_{2u} globulin are demonstrated in Figure 3, in which it can be seen that the newly synthesized polypeptide immunoprecipitated with anti-α_{2u} migrates with authentic α_{2u} globulin on sodium dodecyl sulfate-polyacrylamide gels, and that [^{3}H]leucine incorporation into α_{2u} globulin is a function of the amount of hepatic mRNA added. Parallel saturation of [^{3}H]leucine incorporation into

[2] A. Sippel, D. T. Kurtz, and P. Feigelson, manuscript in preparation.

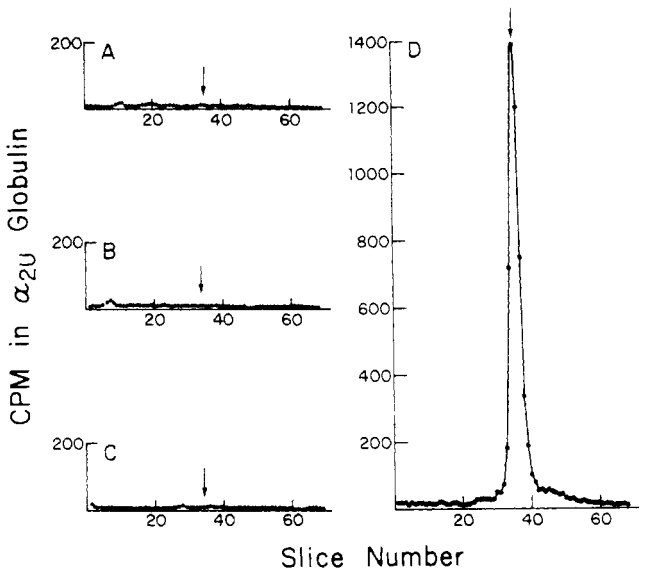

FIGURE 4: Sodium dodecyl sulfate-polyacrylamide gel electrophoretic profiles of α_{2u} globulin synthesized in vitro by mRNA from male rat liver, female rat liver, and male rat kidney. Protein synthesis performed as described under Materials and Methods. α_{2u} globulin immunoprecipitated from 400 μl of released chain fraction of the WG S30 system containing: (A) no exogenous mRNA, or 100 μg of mRNA from (B) female liver, (C) male kidney, and (D) male liver.

protein occurs with respect to α_{2u} globulin and total protein synthesis (Figure 2).

It has been shown (Roy and Neuhaus, 1966b) that female rats produce no α_{2u} globulin, and that, in the male rat, synthesis of α_{2u} globulin occurs only in the liver. Poly(A)-containing RNA, extracted from the livers of female rats and from the kidneys of male rats, was translated in the WG S30 system. These mRNA species directed [^{3}H]leucine incorporation into total and released protein at levels comparable to that directed by male rat liver mRNA, but did not direct the synthesis of any immunologically identifiable α_{2u} globulin (Figure 4). This demonstrates that the peaks seen in Figure 3 are not due to nonspecific immunoprecipitation or nonspecific binding of [^{3}H]leucine to the immunoprecipitate.

α_{2u} Globulin Levels in Serum and Liver Cytosol. Because the urinary output of α_{2u} globulin was found to vary greatly among rats of the same age and body weight (from 4.0 to 15 mg of α_{2u}/24 h), and also varied by as much as 30% for the same rat for two consecutive 24-h periods, a precise control value of urinary α_{2u} globulin excretion was impossible to define. Therefore, immunologically determined α_{2u} globulin concentrations in serum and liver S100 were used to monitor α_{2u} globulin repression or induction under the various hormonal manipulations. Radial immunodiffusion measurements indicated α_{2u} globulin to be 0.1% of total hepatic S100 protein (i.e., 1 μg of α_{2u}/mg of protein) in a normal adult male rat. Serum α_{2u} globulin was present at a concentration of 0.12 μg of α_{2u}/mg of protein, approximately 1 mg %. These values varied only ±5% for control males. In endocrine deficiency states, when α_{2u} globulin was present in low levels in the serum or liver S100, it could be measured by lyophilizing the sample and redissolving in a smaller volume. Using this procedure, the concentration of α_{2u} globulin in serum and liver S100 could be measured down to 20 and 5%, respectively, of the control values.

Effect of Thyroid Hormones on α_{2u} Globulin Synthesis.

Table II: Control of α_{2u} Globulin Synthesis and α_{2u} Globulin mRNA by Thyroid Hormones.

Endocrine State	Serum α_{2u}[a] (% of Control)	Liver S100 α_{2u}[a] (% of Control)	In Vivo Labeling of α_{2u}[b] (% of Control)	α_{2u} mRNA[c] (% of Control)
Thyroidectomized	0	0	0	0
Thyroidectomized + 4 days T4	<20	7.5		20.1
Thyroidectomized + 10 days T4	92.1	97.5		105
Thyroidectomized + 2 days T3	<20	13.3		17.5
Thyroidectomized + 4 days T3	31.8	35.9	30.2	35.3
Tx + DHT or androsterone or androstenedione	0	0		0

[a] Serum and liver S100 α_{2u} globulin concentrations were determined by the radial immunodiffusion assay. Control values are: serum, 0.12 mg of α_{2u}/mg of protein; liver S100, 1.05 mg of α_{2u}/mg of protein. [b] In vivo labeling determined as: (counts per minute in a α_{2u} globulin)/(counts per minute in total Cl_3CCOOH-precipitable protein) in 1 ml of liver cytosol from rats given intraperitoneal injections of [^{3}H]leucine 15 min prior to sacrifice. The counts per minute in α_{2u} globulin was determined by summing the counts in the peaks shown in Figure 5. The counts per minute in total protein was determined by precipitating a 10-μl aliquot of the labeled liver cytosol with Cl_3CCOOH, washing the precipitate with Cl_3CCOOH, and dissolving the precipitate and subjecting it to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in parallel with gels containing the washed α_{2u} globulin immunoprecipitates. After electrophoresis, these total protein gels were sliced and counted, and the total counts on the gels were summed. In control males, α_{2u} globulin represented 0.96% of hepatic protein synthesis. [c] α_{2u} globulin mRNA was quantitated by determining the counts per minute incorporated into α_{2u} globulin in vitro by summing the counts per minute in the α_{2u} globulin peaks (such as those seen in Figure 6). The counts were normalized to counts per minute incorporated into α_{2u} globulin (per 10^6 cpm incorporated) into released polypeptide chains.

As reported, thyroidectomy abolishes α_{2u} globulin synthesis. No α_{2u} globulin could be detected in the sera or liver cytosols of thyroidectomized male rats (Table II). Furthermore, thyroidectomized males showed no incorporation of [^{3}H]leucine into α_{2u} globulin in vivo (Figure 5). Administration of T3 or T4 restored α_{2u} globulin synthesis, as measured in serum and liver cytosol (Table II), and in vivo incorporation of [^{3}H]leucine into α_{2u} globulin (Figure 1 and Table II). α_{2u} globulin could be detected in the serum and liver S100 after 2 days of hormone administration, and reached control levels by 10 days of treatment.

Effect of Thyroid Hormones on α_{2u} Globulin mRNA. To determine if this modulation of α_{2u} globulin synthesis by thyroid hormones is the result of control of the functional level of its mRNA, the hepatic poly(A)-containing RNA was extracted from the livers of the thyroidectomized and hormone treated males, and translated in the wheat germ cell-free protein synthesizing system. The released polypeptide chains synthesized in vitro were collected and immunoprecipitated with anti-α_{2u} to quantitate the functional level of α_{2u} globulin mRNA. Thyroidectomized males had no detectable α_{2u} globulin mRNA (Figure 6). Administration of T4 or T3 resulted in the production of functional mRNA for α_{2u} globulin (Figure 6 and Table II). The time course of the induction of α_{2u} globulin mRNA by thyroid hormones paralleled the appearance of the protein in serum and liver cytosol, and the increase of [^{3}H]leucine incorpora-

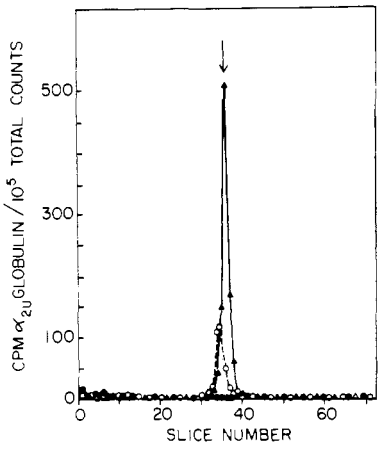

FIGURE 5: Sodium dodecyl sulfate-polyacrylamide gel electrophoretic profiles of α_{2u} globulin synthesized in vivo by control, thyroidectomized, and thyroid hormone-treated males. Rats were sacrificed 15 min after intravenous injection of [^{3}H]leucine (1 mCi/100 g), and liver cytosols prepared as described under Materials and Methods. The α_{2u} globulin content of the cytosols was determined, and anti-α_{2u} IgG was added to 1 ml of the cytosols to give antigen-antibody equivalence. (For the immunoprecipitation of the thyroidectomized rat liver cytosol, where no α_{2u} globulin was detectable, 10 μg of carrier α_{2u} globulin was added before the addition of antibody). The immunoprecipitates were washed thoroughly as described, dissolved, and electrophoresed on 0.1% sodium dodecyl sulfate-10% polyacrylamide gels. α_{2u} globulin immunoprecipitated from livers of control males (▲—▲), thyroidectomized males (●—●), and thyroidectomized males after 4 days treatment with T3 (○ - - - ○). Arrow marks position of authentic α_{2u} globulin marker.

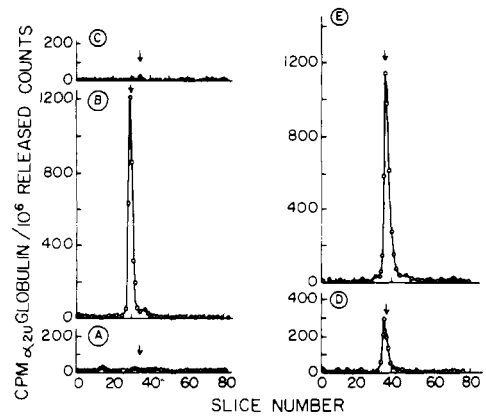

FIGURE 6: Sodium dodecyl sulfate-polyacrylamide gel electrophoretic profiles of α_{2u} globulin synthesis in vitro directed by liver mRNA from control, thyroidectomized, and thyroid hormone-treated males. Protein synthesis was performed as described under Materials and Methods. α_{2u} globulin immunoprecipitated from 400 μl of the released chain fraction of the WG S30 system containing: (A) no exogenous mRNA, or 100 μg of mRNA from the livers of: (B) control males, (C) thyroidectomized males, (D) thyroidectomized males after 4 days treatment with T4, and (E) thyroidectomized males after 10 days treatment with T4. Arrows mark position of authentic α_{2u} globulin marker.

tion in vivo into α_{2u} globulin (Table II).

Effect of Androgens on α_{2u} Globulin Synthesis in Thyroidectomized Males. It was reported by Hellman et al. (1959) that the level of thyroid hormones greatly influences the production of androgens in male rats. Since androgens play a primary role in the regulation of α_{2u} globulin synthesis (Roy and Neuhaus, 1967; Sippel et al., 1975), the possibility existed that the observed effect of thyroid hormones on α_{2u} globulin synthesis in thyroidectomized males was the result of a decrease in androgen production following thyroidectomy. To test this possibility, thyroidectomized male rats were given daily injections of dihydrotestosterone or androstenedione or androsterone for 4 days. After such treatment, no α_{2u} globulin was detectable in sera or liver cytosols, and no α_{2u} globulin mRNA was found in the livers from these androgen-treated, thyroidectomized males (Table II). It would appear, therefore, that the effect of thyroid hormones on α_{2u} globulin synthesis is not a result of modulation of androgen levels.

Discussion

Previous studies using this method of in vitro translation and immunoprecipitation to quantitate tissue mRNA levels for specific proteins, such as ovalbumin (O'Malley et al., 1972), casein (Houdebeine and Gaye, 1975), silk fibroin (Greene et al., 1975), or globin (Palmiter, 1973), have dealt with proteins which constitute a high percentage of the total protein synthesis in the particular tissue of origin. Studies using a wheat germ cell-free protein synthesizing system to study the in vitro synthesis of specific proteins have also dealt with proteins which represent a relatively large percentage of total protein synthesis, e.g., tubulin and actin (Gozes et al., 1975), which represent 25 and 10%, respectively, of total protein synthesis in 10-day-old rat brain. Using the techniques described in this paper, it is possible to measure accurately the functional level of the hepatic mRNA for α_{2u} globulin, a protein under complex hormonal control, which represents approximately 1% of the total hepatic protein synthesis in vivo in the male rat.

The results presented here are the first indication that thyroid hormones may influence the synthesis of a specific protein by controlling the functional level of the mRNA coding for that protein. The results obtained earlier in this laboratory (Sippel et al., 1975) using a Krebs II ascites translational system to monitor α_{2u} globulin mRNA levels also indicated such pretranslational control of α_{2u} globulin synthesis by sex steroids, glucocorticoids, and pituitary hormones. In all instances, a good correlation was found between the functional level of the mRNA for α_{2u} globulin, as assayed in the translational systems, and the in vivo rate of synthesis of α_{2u} globulin, as well as the hepatic, serum, and urinary levels of this protein. It would appear, therefore, that the primary mechanism for the hormonal regulation of α_{2u} globulin synthesis involves the control of the level of the mRNA coding for this protein. These results are consistent with a model involving direct gene effects by sex steroids, glucocorticoids, pituitary hormones, and thyroid hormones in modulating α_{2u} globulin biosynthesis. Such a model is consistent with the nuclear thyroid hormone receptor studies mentioned in the introductory statement, and would suggest that thyroid hormone action may follow the pattern established for the action of steroid hormones on their target tissues (Feigelson et al., 1962, 1975; Jensen and DeSombre, 1972). However, no firm conclusions can be drawn as to whether direct transcriptional control is, in fact, the mode of action of these hormones in regulating the functional level of the mRNA for the hormone-inducible proteins. We cannot exclude the possibility that the hormonal modulation of mRNA levels is the result of control of nuclear or cytoplasmic processing and transport of the mRNA. Hormonal control of mRNA stability, however, seems improbable in view of recent findings (Killewich et al., 1975).

The results presented here do unambiguously indicate that thyroid hormones control α_{2u} globulin synthesis by acting pretranslationally, and not indirectly through the androgens. The questions concerning direct vs. indirect effects of

thyroid hormones on α_{2u} globulin synthesis become more complex in light of a recent report by Hervas et al. (1975), in which it was shown that L-thyroxine and triiodo-L-thyronine can affect the levels of growth hormone in rats. Definitive resolution of these complexities awaits the development of an in vitro hormone-responsive α_{2u} globulin synthesizing system. Such hepatocyte systems are presently under development.

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