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Alterations in albumin secretion and total protein synthesis in livers of thyroidectomized rats

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Perfused rat livers and isolated rat hepatocytes exhibited a 50% decrease in the secretion of both albumin and total secretory proteins after thyroidectomy. In contrast, synthesis of non-secretory proteins was decreased by only 20% from the rates observed in liver preparations from euthyroid rats. These observations suggested a disproportionate effect of thyroidectomy on the synthesis of secretory proteins compared with non-secretory proteins. Disproportionate decreases in the synthesis of albumin in other endocrine-deficient states such as hypophysectomy and diabetes had previously been shown to be associated with decreases of similar magnitude in the relative abundance of albumin-mRNA sequences. In contrast, thyroidectomy did not affect the activity or amount of albumin mRNA in total liver poly(A)-containing RNA when assayed by cell-free translation and by hybridization with complementary DNA, respectively. Furthermore, labelling experiments *in vivo* demonstrated that albumin synthesis represented $12.9 \pm 0.5\%$ and $12.4 \pm 0.4\%$ of total protein synthesis in livers of thyroidectomized and euthyroid rats respectively. Therefore the fall in secretion of albumin and total secretory protein after thyroidectomy did not appear to be a reflection of disproportionate decreases in the synthesis of these proteins. Instead, defects in steps involved in the post-synthetic processing and secretion of albumin are suggested. A number of comparisons, including ribosome half-transit times, the size distributions of total and albumin-synthesizing polyribosomes, and the fraction of RNA present as inactive ribosomes, provided evidence that the overall decrease in protein synthesis after thyroidectomy was not due to generalized alterations in translational processes. Instead, the decrease in total protein synthesis appeared to reflect the RNA content of the liver, which fell in proportion to the decrease in protein synthesis.

The present study was undertaken to compare the effects of thyroidectomy on liver protein synthesis with those of other endocrine-deficient states such as hypophysectomy and diabetes. Such a comparison was considered important because of the known multiple hormonal alterations that occur in thyroidectomized, hypophysectomized and diabetic rats. For instance, serum somatotropin (growth hormone) concentrations are markedly decreased in thyroidectomized rats (Coiro *et al.*, 1979), presumably because of a direct effect of tri-iodothyronine to regulate the somatotropin-mRNA content of the pituitary (Martial *et al.*, 1977; Seo *et al.*, 1977). Furthermore, circulating thyroid-hormone concentrations are decreased in diabetes (Zaninovich *et al.*, 1977; Gonzalez *et al.*, 1980), and circulating insulin is decreased in both thyroidectomized (Jolin *et al.*, 1970) and hypophysectomized (Ball & Knobil, 1963) animals. Thus the possibility existed that observed effects of endocrine ablation on liver protein synthesis were not due to the primary endocrine deficiency, but instead were the result of a secondary hormonal change.

For two reasons our attention was focused on albumin, the major protein-synthesis product of the liver. First, we (Peavy *et al.*, 1981) and others (Griffin & Miller, 1973) had previously observed that thyroidectomy results in a relatively greater impairment in plasma protein secretion than is observed for overall liver protein synthesis. Second, we had previously observed that albumin synthesis is affected to a greater extent than is the synthesis of

Abbreviations used: Pipes, 1,4-piperazinediethanesulphonic acid; poly(A)$^+$ RNA, poly(A)-containing RNA; cDNA, complementary DNA.

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total liver protein after either hypophysectomy (Feldhoff *et al.*, 1977) or the induction of diabetes (Peavy *et al.*, 1978). In hypophysectomized rats, a 70% decrease in albumin secretion was paralleled by a similar fall in total plasma protein secretion, and was the result of a decrease in the relative number of albumin-mRNA sequences (Feldhoff *et al.*, 1977; Keller & Taylor, 1979). In contrast, synthesis of non-export proteins was decreased by only 40% after hypophysectomy (Feldhoff *et al.*, 1977). In rats with experimental diabetes, albumin secretion was decreased by 90% from normal values, in comparison with a 75% decrease in total plasma protein secretion and a 55% decrease in the synthesis of non-export proteins (Peavy *et al.*, 1978). Again, the lower albumin secretion was the result of a decrease in the relative abundance of albumin mRNA (Peavy *et al.*, 1978).

Since thyroidectomy results in an overall decrease in the synthesis of liver proteins, we also decided to investigate the possibility of alterations in translational processes. Previous reports had suggested that thyroid hormones might influence the processes of both peptide-chain elongation and initiation. Mathews *et al.* (1973) reported that rates of peptide-chain elongation in livers of thyroidectomized rats were decreased by 65% compared with euthyroid rats, and were restored to normal values by treatment of thyroidectomized animals with tri-iodothyronine. On the other hand, Tipton (1969) found polyribosomes to be disaggregated in livers of thyroidectomized rats in comparison with euthyroid controls, which suggested that the rate of peptide-chain initiation was slowed to a greater extent than was the rate of peptide-chain elongation.

Our findings demonstrate that the disproportionate fall in the secretion of albumin after thyroidectomy is not due to a preferential decrease in the synthesis of this protein, but instead appears to reflect an impairment in post-synthetic processing or secretory mechanisms. In addition, our findings do not reveal any effects of thyroidectomy on the translational processes of peptide-chain elongation and initiation. Instead, the effect of thyroidectomy to decrease total hepatic protein synthesis parallels and is probably the result of the loss of tissue RNA.

Experimental

Animals

Normal and thyroidectomized rats of the Sprague–Dawley strain were supplied by Charles River Breeding Laboratories (Wilmington, MA, U.S.A.). Animals weighing 55–57g were surgically thyroidectomized and were used in experiments when little further weight gain was evident, usually 4–8 weeks after surgery, at which time they weighed approx. 160g. Euthyroid control rats also weighed approx. 160g at time of use. All animals were maintained on a 12h-light/12h-dark cycle and were allowed free access to food (Purina Lab Chow or Wayne Lab Blox) and water at all times.

Liver perfusions

Liver perfusions *in situ* were performed as described previously (Peavy *et al.*, 1981) with a flow rate of 7ml/min and a recirculating volume of 100ml. The perfusion medium consisted of Krebs–Henseleit (1932) bicarbonate buffer (pH 7.4) containing 10mM-glucose, 3% (w/v) bovine serum albumin (Pentex bovine albumin, Fraction V; Miles Laboratories, Elkhart, IN, U.S.A.), amino acids at ten times the concentrations found in normal rat plasma (Tolman *et al.*, 1973), and sufficient bovine erythrocytes to give a haematocrit of 30%. To enable the determination of accurate rates of protein synthesis, [4,5-^{3}H(n)]leucine (New England Nuclear, Boston, MA, U.S.A.) was added to the perfusate to give a final concentration of 5mM. It was previously shown that perfusion of livers with a high concentration of labelled amino acid results in rapid equilibration of the specific radioactivity of aminoacyl-tRNA to a value equal to that of the amino acid in the intracellular and extracellular compartments (Khairallah & Mortimore, 1976; K. E. Flaim, D. E. Peavy & L. S. Jefferson, unpublished work), thus permitting the use of the specific radioactivity of the extracellular amino acid for accurate determination of rates of protein synthesis (Feldhoff *et al.*, 1977).

Samples of the perfusate were collected during the course of 120min of perfusion, centrifuged at $1000g_{av.}$ for 10min to remove erythrocytes, and frozen for subsequent analysis. At the end of perfusion, livers were rapidly removed from the carcass, blotted, weighed, and frozen in aluminium tongs precooled to the temperature of liquid nitrogen. Perfusate samples were assayed for incorporation of [^{3}H]leucine into total secreted protein by trichloroacetic acid precipitation or into albumin by immunoprecipitation as described previously (Taylor & Schimke, 1973). Liver samples were assayed for incorporation of label into those proteins remaining in the liver (intracellular or non-secretory proteins) by trichloroacetic acid precipitation (Peavy *et al.*, 1981). Efficiency of radioactivity counting was approx. 40% and quench correction was done by an external-standard method. Rates of protein synthesis for each of the protein fractions were calculated as described previously (Peavy *et al.*, 1981).

Hepatocyte preparation

Isolated hepatocytes were prepared from livers of both normal and thyroidectomized rats by perfusion with collagenase as described previously (Feldhoff *et al.*, 1977). After digestion with the enzyme, hepatocytes were collected and washed by a

sedimentation/resuspension procedure. After the final wash, the cells were resuspended in 20 vol. of bicarbonate buffer (Feldhoff *et al.*, 1977) containing 5mM- or 0.16mM- (the normal plasma concentration) leucine and all other amino acids at 7.5 times the normal rat plasma concentrations (Tolman *et al.*, 1973). The cell suspensions were preincubated for 30min at 37°C in a shaking water bath (rotary motion, 150rev./min) before the start of an experiment, and cell viabilities were typically in the range 90–95%. All wash and incubation buffers were maintained at 37°C and equilibrated with O_2/CO_2 (19:1). After the preincubation period, cells were transferred to 10 or 40 vol. of fresh incubation medium containing labelled leucine. For measurements of protein synthesis, the medium contained 5mM-[^{3}H]leucine (5μCi/ml), the cells were diluted 1:40, and the incubations were carried out for 4h. Samples of the incubation medium and isolated cell pellets were assayed for incorporation of [^{3}H]leucine into albumin, total secretory proteins and non-secretory proteins as described above for liver-perfusion experiments. For determination of half-transit times, the medium contained 0.16mM-[^{3}H]-leucine (50μCi/ml) and the cells were diluted 1:10. At various time points, samples of the cell suspension were homogenized at 4°C in 50mM-Tris/HCl buffer, pH7.6, containing cycloheximide (10μg/ml), Triton X-100 (1%, v/v) and sodium deoxycholate (1%, w/v). The homogenate was centrifuged in a Sorvall HB-4 rotor at 10500$g_{av.}$ for 10min, and portions of the supernatant were further centrifuged in a Beckman SW 60 rotor at 320000$g_{av.}$ for 120 min to pellet polyribosomes. Incorporation of radioactivity into total protein or albumin was determined in both the 10500$g_{av.}$ and postribosomal supernatants as described previously (Feldhoff *et al.*, 1977).

Isolation of poly(A)$^+$ RNA

Total rat liver RNA was prepared from unfractionated liver homogenates by phenol/chloroform extraction (Keller & Taylor, 1976), and the poly(A)$^+$ RNA isolated by affinity chromatography on poly(U)–Sepharose as described previously (Taylor & Tse, 1976). Sucrose-gradient analysis of the RNA indicated that essentially all rRNA was removed. A value of 1 A_{260} unit/50μg of RNA (Taylor & Tse, 1976) was employed to calculate the concentration of RNA in all samples. The poly(A)$^+$ RNA samples had an A_{260}/A_{280} ratio of 2.2–2.3.

Cell-free translation assay of albumin-mRNA activity

Poly(A)$^+$-RNA samples were examined for total and albumin-mRNA translational activity by using mRNA-dependent rabbit reticulocyte lysates. Lysates were made mRNA-dependent by pre-treatment with micrococcal nuclease as described by Pelham & Jackson (1976). The translation assay was performed as described previously (Peavy *et al.*, 1978), and the incorporation of [^{3}H]leucine into albumin or total protein was determined as described by Taylor & Schimke (1973).

Hybridization assay of albumin mRNA

The relative amount of albumin mRNA was also determined by hybridization of excess poly(A)$^+$ RNA to a specific ^{3}H-labelled cDNA prepared to rat albumin mRNA as described by Keller & Taylor (1977). Hybrid formation was determined by assaying the reaction samples for S_1-nuclease-resistant radioactive material (Tse *et al.*, 1978).

Albumin synthesis in vivo

Relative rates of albumin synthesis *in vivo* were determined as described previously (Peavy *et al.*, 1978). Briefly, animals were given an intraperitoneal injection of 500μCi of [^{3}H]leucine, and 10min later (before secretion of labelled protein would occur) the liver was removed and homogenized in 3vol. of 250mM-sucrose. Then 0.1vol. of a 10% (v/v) Triton X-100/10% (w/v) deoxycholate solution was added to release microsomal proteins, and the homogenate was then briefly centrifuged to remove debris. Radioactivity in albumin was determined by immunoprecipitation with a specific antibody and the radioactivity in total protein was determined by trichloroacetic acid precipitation (Taylor & Schimke, 1973). Radioactivity in albumin relative to the radioactivity in total protein was then calculated.

Isolation of polyribosomes

Method I. Rat livers were homogenized at 4°C in 6vol. of Buffer A (40mM-Pipes/100mM-KCl/5mM-$MgCl_2$, pH6.5) containing 250mM-sucrose, 5mM-glutathione (reduced form) and heparin (0.75mg/ml; sodium salt) by using a motor-driven Teflon/glass homogenizer, and were adjusted to 1% (v/v) Triton X-100/1% (w/v) sodium deoxycholate. The detergent-treated homogenate was centrifuged at 10500$g_{av.}$ at 4°C for 10min in a Sorvall HB-4 rotor, and the supernatant fluid was decanted. Portions of the supernatant fluid were overlaid on discontinuous sucrose gradients consisting of 2ml of 2.5M-sucrose, 4ml of 1.0M-sucrose and 0.6ml of 0.5M-sucrose, each in Buffer A containing heparin (0.5mg/ml; sodium salt). In addition, the 0.5M-sucrose layer contained 5mM-glutathione. The gradients were centrifuged in a Beckman SW41 rotor at 210000$g_{av.}$ for 100min at 4°C and the polyribosomes sedimenting to the 1.0M/2.5M-sucrose interface were collected and dialysed overnight at 4°C against Buffer B (50mM-Tris/HCl/25mM-NaCl/5mM-$MgCl_2$, pH7.65) containing heparin (0.2mg/ml; sodium salt).

Method II. Livers were homogenized in the medium described in Method I, to which additional heparin was added to give a final concentration of 5 mg/ml and which was supplemented with 1% (v/v) diethyl pyrocarbonate and 2.7 mg of phenol-extracted yeast RNA/ml, as described by Jost *et al.* (1978). The polyribosome isolation was then continued as described in Method I.

Binding of anti-albumin antibodies to polyribosomes

Albumin antibodies were immunopurified by affinity chromatography as described previously (Taylor & Schimke, 1974). The purified antibodies were labelled with ^{125}I ($Na^{125}I$, 2.5 Ci/mol; New England Nuclear) by means of lactoperoxidase coupled to microspheres (Enzymobeads; Bio-Rad Laboratories, Richmond, CA, U.S.A.) according to the manufacturer's directions and were repurified by gel filtration and ion-exchange chromatography to remove ribonucleases (Taylor & Schimke, 1974). A sample (10 μg) of the labelled antibody preparation (2.07×10^5 c.p.m./μg) was incubated with various amounts of polyribosomes in a volume of 1 ml at 4°C for 30 min before examination on linear 15–50% (w/v) sucrose gradients prepared in Buffer B containing heparin (150 μg/ml). The gradients were centrifuged in a Beckman SW41 rotor at 210000 $g_{av.}$ for 100 min at 4°C, and the A_{260} profile of the gradients was monitored and 0.6 ml fractions were collected to determine the ^{125}I distribution on the gradient.

Results

Protein synthesis

The effect of thyroidectomy on hepatic protein synthesis was evaluated in experiments with perfused livers and isolated hepatocytes (Fig. 1). In these experiments, samples of the perfusion medium or incubation medium, and of the liver or isolated cell pellets, were taken to determine rates of production of secretory and non-secretory proteins respectively. Thyroidectomy resulted in an approx. 50% decrease in the secretion of both albumin and total secretory proteins, whereas a 20% decrease in the synthesis of non-secretory proteins was observed. Similar effects were seen in both perfused livers and isolated hepatocytes. These results confirmed previous studies indicating that thyroidectomy results in a disproportionate decrease in the secretion of albumin and total secretory proteins compared with the effect on total liver protein synthesis (Griffin & Miller, 1973; Peavy *et al.*, 1981).

Determination of albumin mRNA

Previous studies had shown that both hypophysectomized and diabetic rats exhibited disproportionate decreases in hepatic albumin secretion relative to the changes seen in total liver protein synthesis (Feldhoff *et al.*, 1977; Peavy *et al.*, 1978). In both cases, the decreases in albumin secretion were due to falls of similar magnitude in the relative abundance of albumin-mRNA sequences. To test the possibility that a similar alteration might explain

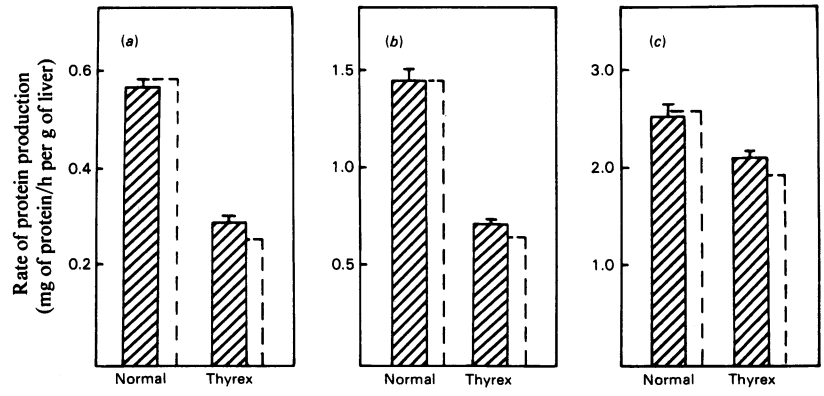

Fig. 1. *Effects of thyroidectomy on protein secretion and synthesis in perfused rat livers and isolated hepatocytes*
Livers were perfused *in situ* for 2 h and hepatocytes were incubated for 4 h with buffer containing 5 mM-[^{3}H]leucine as described in the Experimental section. Samples of the perfusion or incubation medium were taken for determination of rates of albumin (*a*) or total protein (*b*) secretion, and samples of liver tissue or cell pellets were taken for determination of synthesis of non-secretory liver proteins (*c*). Results from liver perfusion experiments (shaded bars) are the means ± S.E.M. for six or more determinations. The offset broken lines depict results obtained with isolated hepatocytes and represent the means of three experiments. Standard errors were similar in magnitude to those observed in liver-perfusion experiments. In each case, the rate observed in tissue from thyroidectomized animals (Thyrex) was significantly different ($P < 0.01$, Student's t test) from that in the corresponding tissue preparation from normal animals (Normal).

the decrease in albumin secretion observed in livers of thyroidectomized rats, total liver poly(A)$^+$ RNA was isolated and the relative abundance of albumin mRNA was assessed in cell-free translation and cDNA-hybridization assays. The cell-free translation assay employed was the mRNA-dependent reticulocyte-lysate system (Pelham & Jackson, 1976). The results (Fig. 2) show that relative albumin-mRNA translational activity was the same regardless of whether the poly(A)$^+$ RNA was derived from livers of normal or thyroidectomized rats. The relative abundance of specific albumin mRNA sequences of the poly(A)$^+$ RNA samples was also measured by RNA-excess hybridization to a specific cDNA probe (Fig. 3). Identical results were obtained with RNA from control and thyroidectomized rats, indicating that the relative abundance of albumin mRNA sequences in hepatic poly(A)$^+$ RNA was unaltered by thyroidectomy. Thus, in contrast with the situation in livers of hypophysectomized or diabetic rats (Keller & Taylor, 1979); Peavy *et al.*, 1978), the disproportionate decrease in albumin secretion by perfused livers of thyroidectomized animals was not the result of an alteration in the relative abundance of albumin mRNA.

Relative rate of albumin synthesis in vivo

Although results of experiments employing liver perfusion and isolated hepatocytes clearly indicated a disproportionate decrease in albumin secretion by livers of thyroidectomized rats, the data pertaining to the relative abundance of albumin mRNA suggested the likelihood of an unaltered rate of albumin synthesis relative to total protein synthesis in this situation. Relative rates of albumin synthesis were therefore determined in experiments in which the incorporation of [^{3}H]leucine into both albumin and total liver protein was determined after a 10min

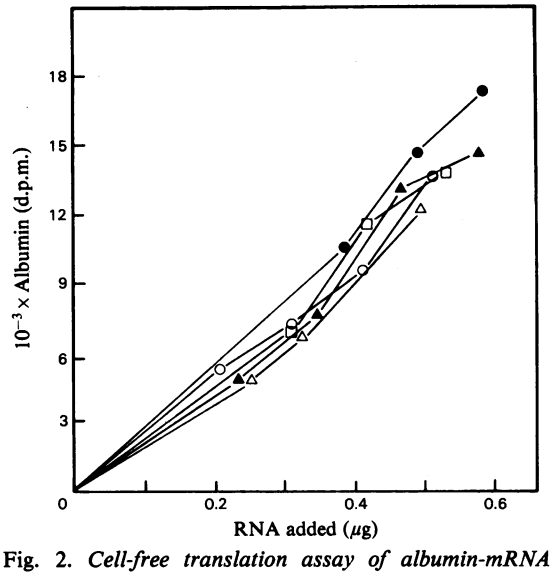

Fig. 2. *Cell-free translation assay of albumin-mRNA activity*

Total liver poly(A)$^+$ RNA was assayed for albumin-mRNA activity by using rabbit reticulocyte lysates pre-treated with micrococcal nuclease as described in the Experimental section. The incorporation of [^{3}H]leucine into immunoprecipitable albumin is expressed relative to the amount of poly(A)$^+$ RNA added to the reaction mixture. Each symbol represents RNA from pooled liver samples from two or three rats. Representative results from two separate assays are shown. Solid symbols, RNA isolated from control rats; open symbols, RNA isolated from thyroidectomized rats.

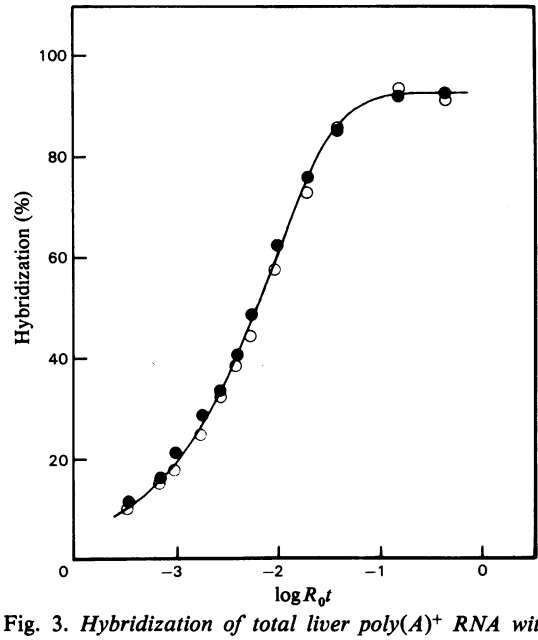

Fig. 3. *Hybridization of total liver poly(A)$^+$ RNA with albumin cDNA*

Total poly(A)$^+$ RNA was hybridized up to 2500-fold weight excess to 100pg of albumin cDNA (470 nucleotides in length). Hybrid formation was determined by resistance to S_1 nuclease. R_0t is the product of RNA concentration (mol of nucleotide/litre) and the hybridization time (s). Hybridization analyses were repeated three times with RNA prepared from livers pooled from two or three rats. Each of the poly (A)$^+$ RNA preparations gave equivalent results, and therefore only representative examples are shown. A $R_0t_{\frac{1}{2}}$ of 5.62×10^{-3} mol·s·l^{-1} (R_0t value at which the hybridization was 50% complete) was observed with RNA from livers of both control (●) and thyroidectomized rats (○).

labelling period *in vivo*. Because labelled protein is not secreted within the 10min labelling period (Peters, 1962) and the leucine content of albumin is similar to that of total hepatic protein (Peters, 1975; Schreiber *et al.*, 1971), radioactivity in albumin relative to that in total protein approximates the relative rate of albumin synthesis. In livers of thyroidectomized animals, albumin synthesis represented $12.9 \pm 0.5\%$ (mean $\pm$ S.E.M.) of total protein synthesis, which was not significantly different from the value of $12.4 \pm 0.4\%$ observed in livers of normal control animals. Similar values for the relative rate of albumin synthesis were also obtained when this type of labelling experiment was conducted in perfused livers or isolated hepatocytes from normal or thyroidectomized rats (D. E. Peavy, unpublished work). Therefore the disproportionate decrease in albumin secretion observed in perfused livers of thyroidectomized animals was not the result of a selective decrease in albumin synthesis. Instead, the impairment in secretion must result from a defect in post-synthetic events involved in processing and/or secretion of albumin and other secretory proteins.

Ribosome half-transit time

Examination of tissue RNA concentrations revealed that thyroidectomy resulted in similar decreases in tissue RNA and protein synthesis. Total hepatic RNA was decreased from 7.83 ± 0.14 mg/g wet wt. of liver (mean $\pm$ S.E.M.) in normal animals to 5.82 ± 0.10 mg/g in thyroidectomized animals (a 26% decrease). Total protein synthesis was decreased by 20% after thyroidectomy, assuming that

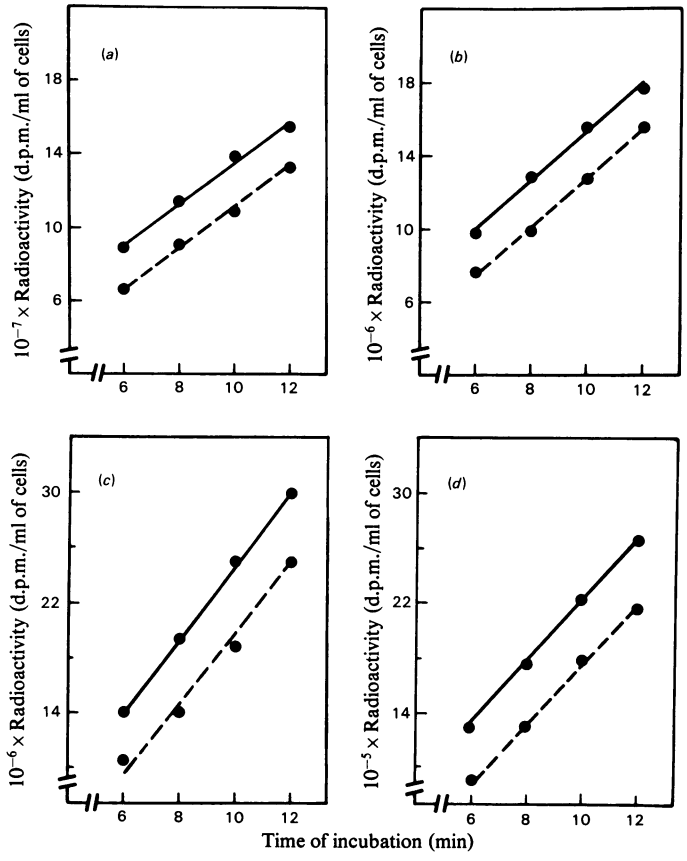

Fig. 4. *Determination of ribosome half-transit times ($t_{\frac{1}{2}}$) in isolated hepatocytes prepared from normal and thyroidectomized rats*

Isolated hepatocytes were prepared and incubated as described in the Experimental section. At the indicated time points a sample of the cell suspension was homogenized and 10500*g* (●——●) and 320000*g* (●- - - -●) supernatant fluids were prepared. Incorporation of [^{3}H]leucine into either total protein (*a*, *c*) or albumin (*b*, *d*) in each of the supernatant fluids was determined. The displacement in time between the two plotted data lines, which represents the average ribosome half-transit time, $t_{\frac{1}{2}}$ (Fan & Penman, 1970), averaged 1.90 ± 0.10 and 1.90 ± 0.03 min (mean $\pm$ S.E.M., $n = 3$) for cells from normal (*a*) and thyroidectomized (*c*) animals. The respective half-transit times for albumin averaged 1.90 ± 0.10 (*b*) and 1.90 ± 0.10 min (*d*).

the actual fall in secretory protein synthesis was in proportion to the decrease in synthesis of non-export proteins. Thus the overall decrease in protein synthesis in livers of thyroidectomized animals appeared to be the result of a decrease in the capacity of the tissue for protein synthesis. However, several investigators have provided evidence suggesting alterations in translational processes after thyroidectomy as well (Mathews *et al.*, 1973; Tipton, 1969). We therefore examined some of these processes to determine whether such alterations might, in part, be responsible for the differences in total liver protein synthesis that we observed after thyroidectomy. To investigate a possible alteration in peptide-chain elongation, isolated hepatocytes were employed to determine ribosomal half-transit times for albumin and total liver protein by the method of Fan & Penman (1970). The results of these experiments are presented in Fig. 4. According to the Fan & Penman (1970) method, the temporal displacement between the lines depicting incorporation of labelled amino acids into completed plus nascent peptide chains (Fig. 4, solid lines) or into completed peptide chains only (Fig. 4, dashed lines) represents the ribosomal half-transit time. The half-transit times for either albumin (Figs. 4*b* and 4*d*) or total protein (Figs. 4*a* and 4*c*) were the same in hepatocytes from control or thyroidectomized rats. Thus we were unable to confirm that the rate of peptide-chain elongation was altered by thyroidectomy, as previously reported by Mathews *et al.* (1973).

Polyribosome size distribution

Because this distribution reflects a balance between rates of peptide-chain elongation and initiation, we compared the state of aggregation of polyribosomes isolated from livers of normal and thyroidectomized rats. The size distribution of total polyribosomes from livers of normal and thyroidectomized rats prepared by Method I are shown by the solid lines (A_{260}) in Figs. 5(*a*) and 5(*b*), respectively. These profiles show a very large shift in the polyribosome size distribution in livers of thyroidectomized rats as compared with that from euthyroid controls. When equal portions of liver from normal and thyroidectomized rats were homogenized together, however, the size distribution of polyribosomes in this mixture showed changes much like that obtained from livers of thyroidectomized animals alone (results not shown). This finding suggested that the apparent ribosomal disaggregation observed in livers of thyroidectomized rats might be due to ribonucleases released during isolation of polyribosomes. We therefore prepared polyribosomes by using a homogenization buffer formulated to limit more stringently ribonuclease degradation of RNA (Jost *et al.*, 1978). Diethyl

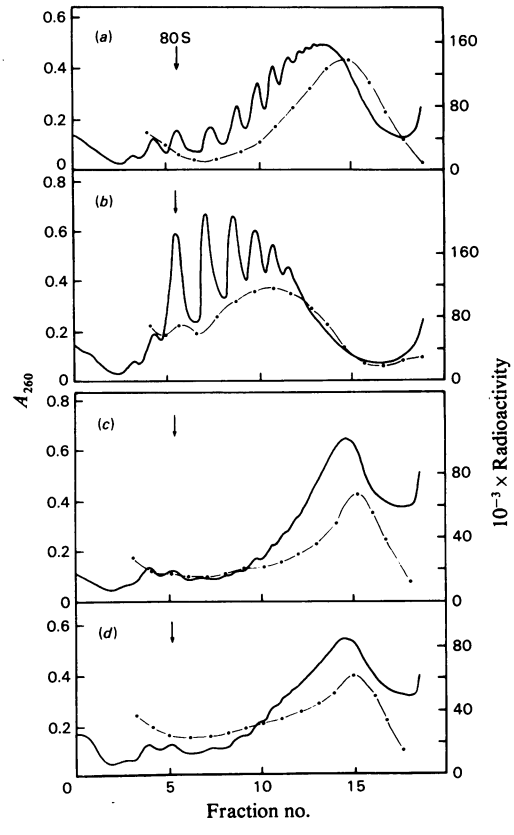

Fig. 5. *Size distribution of total and albumin-synthesizing rat liver polyribosomes*

Total polyribosomes were isolated and a portion (approx. 10 A_{260} units) was analysed on linear 15–50% sucrose gradients as described in the Experimental section. The A_{260} profile of the gradient was determined with a 5mm-path-length flow cell in an ISCO model UA6 flow monitor. The size of albumin-synthesizing polyribosomes was determined by incubating polyribosomes with 10μg of ^{125}I-labelled anti-albumin for 30min at 4°C before examination on sucrose gradients. The distribution of ^{125}I on the gradient was monitored by using an ISCO model 640 density-gradient fractionator to collect 0.6ml fractions. In panels (*a*) and (*b*) polyribosomes were prepared by Method I, and in panels (*c*) and (*d*) they were prepared by Method II (see the Experimental section). (*a*) and (*c*), Polyribosomes from intact control rats; (*b*) and (*d*), polyribosomes from thyroidectomized rats. ——, A_{260}; •——•, anti-albumin radioactivity.

pyrocarbonate and yeast RNA were added to the initial homogenization buffer, and the heparin concentration was increased also (see the Experimental section). The profiles of polyribosomes

prepared under these conditions (Method II) are shown in Figs. 5(*c*) and 5(*d*). Polyribosomes prepared from livers of normal rats (Fig. 5*c*) by this method were slightly larger than those prepared from normal rats by Method I (Fig. 5*a*). The most notable change, however, was in the profile obtained from livers of thyroidectomized rats (Fig. 5*d*). The more rigorous conditions resulted in the isolation of polyribosomes with a size distribution which was essentially the same as that observed with livers of normal animals (compare Figs. 5*c* and 5*d*).

The size and relative number of albumin-synthesizing polyribosomes were determined in these experiments from the binding of ^{125}I-labelled anti-albumin antibodies to polyribosomes through specific recognition of nascent albumin peptide chains (Taylor & Schimke, 1974). The same shift in size of albumin-synthesizing polyribosomes in livers of thyroidectomized rats (Fig. 5*b*, points connected by line) as compared with controls (Fig. 5*a*, points connected by line) was evident when polyribosomes were prepared by Method I; this difference was similarly eliminated by use of the more stringent homogenization buffer (Figs. 5*c* and 5*d*). The relative number of albumin-synthesizing polyribosomes was determined by measuring the total amount of ^{125}I-labelled anti-albumin found in the polyribosome region of the gradient. The results of these determinations are shown in Fig. 6, in which the relative amount of ^{125}I bound is plotted versus the amount of polyribosomes added. The percentage of antibody bound was the same for polyribosomes derived from livers of thyroidectomized animals as compared with controls at each of the polyribosome concentrations examined, and this relationship was evident regardless of which preparation condition was employed to isolate polyribosomes. These results indicated that the relative amount of albumin mRNA present in polyribosomes was not altered by thyroidectomy, which is consistent with the observation described above of unaltered relative rates of albumin synthesis *in vivo* and *in vitro*.

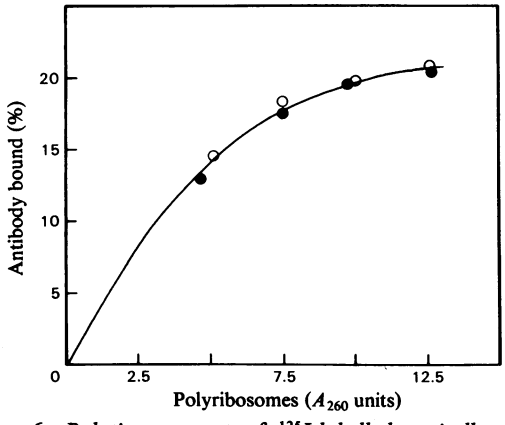

Fig. 6. *Relative amount of ^{125}I-labelled anti-albumin bound to rat liver polyribosomes*

Various amounts of polyribosomes prepared by Method II (see the Experimental section) were incubated with 10μg of ^{125}I-labelled anti-albumin and subjected to sucrose-density-gradient centrifugation as described in Fig. 5. For each polyribosome concentration, the radioactivities in fractions 5–18 inclusive (see Fig. 5) were summed and expressed as a percentage of the total radioactivity of the antibody added. Identical results were observed with polyribosomes prepared by Method I (see the Experimental section). ●, Polyribosomes from intact control rats; ○, polyribosomes from thyroidectomized rats.

Discussion

One purpose of the present study was to evaluate the effects of thyroidectomy on the activity and amount of hepatic albumin mRNA with the intention of understanding the molecular basis for the disproportionate decrease in albumin secretion observed in livers of thyroidectomized rats (Griffin & Miller, 1973; Peavy *et al.*, 1981). Two lines of evidence were available to suggest that a change in the relative amount of albumin mRNA might occur after thyroidectomy. First, thyroid status of the animal was known to influence the relative amount of mRNA coding for α_{2u}-globulin (Kurtz *et al.*, 1976) and 'malic' enzyme (Towle *et al.*, 1980) in rat liver, and tri-iodothyronine was known to cause the induction of specific mRNA coding for somatotropin in a rat pituitary-derived cell line (Martial *et al.*, 1977; Seo *et al.*, 1977). Second, the disproportionate decreases in albumin secretion which occurred in two other endocrine-deficient states, hypophysectomy and diabetes, were known to result from a decrease in the relative abundance of albumin mRNA (Keller & Taylor, 1979; Peavy *et al.*, 1978). The results of the present study demonstrate that, in contrast with the findings described above, the relative amount of albumin mRNA in livers of thyroidectomized rats is not altered from that in livers of euthyroid rats.

The changes observed in albumin secretion do not appear to result from alterations in albumin-mRNA translational activity. This conclusion is supported by several lines of evidence. For example, no differences between the two groups of animals were evident when the size distribution and relative number of albumin-synthesizing polyribosomes were compared, or when albumin peptide-chain-elongation rates were determined. Additionally, the

finding that the relative rate of albumin synthesis *in vivo* or *in vitro* was unchanged after thyroidectomy indicates that albumin is synthesized in proportion to the relative abundance of albumin mRNA in livers of thyroidectomized rats.

Concentrations of poly(A)$^+$ RNA have been reported to parallel changes in total cytoplasmic RNA concentration in livers of control and thyroidectomized rats (Dillmann *et al.*, 1978). Therefore, owing to the 26% decrease in total RNA in livers of thyroidectomized rats that we noted, a similar decrease in poly(A)$^+$ RNA was expected. Since there was not a disproportionate change in albumin mRNA, a 26% decrease in albumin mRNA per g of liver was therefore expected. Thus, about one-half of the 50% decrease in albumin secretion observed after thyroidectomy (Fig. 1) can be explained on the basis of a fall in the absolute amount of albumin mRNA. The remainder of the decrease must result from alterations in steps involved in the post-synthetic processing and secretion of albumin, since no evidence was obtained suggesting alterations in translation of albumin mRNA.

Albumin, like other secretory proteins, is synthesized on polyribosomes attached to the rough endoplasmic reticulum before translocation to the smooth endoplasmic reticulum and Golgi, where it is packaged and processed within secretory vesicles (Munro & Steinert, 1975). Either a deficient mechanism in any of the steps involved in the post-synthetic processing of albumin or co-translational proteolytic degradation could lead to decreased albumin secretion despite apparently normal rates of synthesis. Several examples have been provided indicating alterations in the secretory process for specific proteins. In the Morris hepatoma 5123c, albumin secretion was markedly decreased *in vivo* (Uenoyama & Ono, 1972). This fall was initially thought to be due to an inability of albumin-synthesizing polyribosomes to attach to the rough endoplasmic reticulum (Uenoyama & Ono, 1972), a conclusion based primarily on the finding of nascent albumin peptide chains on free polyribosomes. However, this conclusion was challenged more recently (Redman *et al.*, 1979), as albumin secretion was found to occur normally, although at a much lower rate, in hepatoma 5123c when incubated *in vitro*. Anterior pituitary glands incubated *in vitro* appear to degrade prolactin before secretion, such that the rate of secretion reflects a balance between prolactin synthesis and degradation (Shenai & Wallis, 1979). It was suggested that this may represent a physiologically meaningful mechanism for regulating the tissue concentration of the hormone despite variable rates of secretion (Shenai & Wallis, 1979). Similarly, both parathyrin (Morrissey & Cohn, 1979) and insulin (Halban & Wolheim, 1980) have been shown to be degraded within their cells of origin rather than being quantitatively secreted. Whether the apparent defect in albumin secretion in livers of thyroidectomized rats represents an aberrant condition, as suggested to occur in hepatoma 5123c (Uenoyama & Ono, 1972), or whether it represents a means of regulating tissue concentrations and secretion rates similar to that suggested to occur for hormones such as prolactin, insulin and parathyrin (Shenai & Wallis, 1979; Morrissey & Cohn, 1979; Halban & Wolheim, 1980), remains to be determined. However, in support of the latter possibility, we found that, in spite of an unchanged relative rate of synthesis, intracellular albumin concentrations declined after thyroidectomy in parallel with the decreased rate of secretion. The intracellular albumin content was 534 ± 14, 256 ± 25 and $432 \pm 42 \mu g/g$ of liver (mean $\pm$ S.E.M.) in euthyroid, thyroidectomized and thyroxine-treated thyroidectomized rats, respectively. It is noteworthy that the partial restoration of albumin content in the hormone-treated group was not accompanied by similarly improved secretion rates (Peavy *et al.*, 1981). Although these data suggest the occurrence of intracellular albumin degradation in liver, nothing is at present known about the effect of such a degradative process on albumin secretion in either normal or altered physiological states.

Another purpose of the present study was to investigate possible alterations in translational processes which might account for the fall in overall protein-synthetic rates observed after thyroidectomy. Evaluation of the size distribution of total polyribosomes has provided a useful tool in assessing alterations in protein synthesis under various conditions (Pain, 1978). The usefulness of this approach, however, is largely dependent on the ability to isolate undegraded polyribosomes. By using conditions devised to limit stringently degradation of RNA during isolation of the polyribosomes (Jost *et al.*, 1978), we were able to isolate undegraded polyribosomes from livers of thyroidectomized rats (Fig. 5). Tipton (1969) previously reported a marked shift of liver polyribosomes to smaller sizes after thyroidectomy of donor animals. However, in those studies no precautions were taken to limit degradation of polyribosomes during their isolation. The fact that larger polyribosomes can be isolated from livers of thyroidectomized rats when more potent ribonuclease inhibitors are used suggests greater ribonuclease activity in homogenates from these livers. The similarity of polyribosome sizes between the two conditions suggests that peptide-chain initiation is not different in livers of control and thyroidectomized animals, although co-ordinate changes in rates of peptide-chain initiation and elongation

would not be detected by examining polyribosome sizes alone. In accordance with the finding of similar size distributions of polyribosomes in the two conditions, we have also found identical relative amounts of free ribosomal subunits and monomers in livers of thyroidectomized and euthyroid animals (D. E. Peavy, unpublished work).

Several methods have been described for directly measuring rates of peptide-chain elongation (Fan & Penman, 1970; Haschemeyer, 1969; Palmiter, 1972). Using the approach described by Haschemeyer (1969) to estimate rates of peptide-chain elongation, Mathews *et al.* (1973) reported slower rates of elongation in livers of thyroidectomized rats *in vivo* than in livers of control rats. However, when we compared rates of peptide-chain elongation for albumin and total protein by using the approach of Fan & Penman (1970), rates of elongation of albumin as well as of total tissue protein were not different in hepatocytes taken from thyroidectomized rats or in hepatocytes from control animals. Therefore, because neither polyribosomes sizes nor rates of peptide-chain elongation were different from the control condition, we conclude that rates of peptide-chain initiation and elongation are not altered in livers of thyroidectomized rats. The reason for the disparity between our results and those of Mathews *et al.* (1973) is not readily apparent, but may relate to differences in the model used for study. Maintenance of a constant precursor specific radioactivity is a strict requirement of both the method of Haschemeyer (1969) and that of Fan & Penman (1970). It would be expected that a constant precursor amino acid specific radioactivity would be maintained more closely with isolated hepatocyte suspensions incubated in the presence of a high concentration of precursor, as we used, than after a 10s bolus injection of labelled amino acid *in vivo*, as employed by Mathews *et al.* (1973).

Analysis of the hydridization complexities of poly(A)$^+$ RNA in hypothyroid compared with euthyroid rats (Towle *et al.*, 1979) suggests that thyroidectomy leads to an overall decrease of approx. 30% in all major classes of mRNA. Our findings that thyroidectomy did not alter the size distribution of polyribosomes, the number of polyribosomes relative to the tissue RNA content, or the number of ribosomal subunits or monomers relative to polyribosomes, suggest that the decrease in mRNA is accompanied by simultaneous decreases in other components of the protein-synthetic machinery. Together these changes result in a general decrease in protein synthesis in the hypothyroid state. Thus, except for certain proteins such as α_{2u}-globulin and 'malic' enzyme, whose specific mRNA molecules are preferentially affected (Kurtz *et al.*, 1976; Towle *et al.*, 1980), and possibly other, as yet unidentified, proteins, thyroidectomy appears to lead to a generalized decrease in the liver's capacity to synthesize most classes of proteins.

Finally, results of this study lead to a consideration of the interrelationships among various endocrine deficiencies. Since circulating thyroid hormones are decreased as a result of either hypophysectomy (Taurog *et al.*, 1946) or diabetes (Zaninovich *et al.*, 1977; Gonzalez *et al.*, 1980), a reasonable question to ask was whether the changes in protein synthesis observed in livers of hypophysectomized (Feldhoff *et al.*, 1977) or diabetic rats (Peavy *et al.*, 1978) were due to the hypothyroid status of the animals. The results of the present study clearly demonstrate that thyroid-hormone deficiency was not the primary defect responsible for the alterations in hepatic albumin mRNA which occurred in hypophysectomized (Keller & Taylor, 1979) and diabetic rats (Peavy *et al.*, 1978).

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