teresting in this respect that negative cooperativity has also been reported for a cyclic adenosine 3′:5′-monophosphate phosphodiesterase (Russell et al., 1972). The kinetic data presented in this study and that reported by Russell et al. (1972) are both consistent with either one enzyme under negative cooperative regulation or two separate enzymes. It is clear, however, that either situation provides for substantial regulatory control and suggests that regulation of intracellular cAMP concentrations may be controlled not only by hormones and GTP but also by substrate levels.

Acknowledgments

We are grateful for the excellent technical assistance of Diane Toscano and Nina Cohn. We thank David La Porte and Keith Westcott for useful discussions.

References

Birnbaumer, L., Pohl, S. L., and Rodbell, M. (1972), *J. Biol. Chem. 247,* 2038.
Conway, A., and Koshland, D. E., Jr. (1968), *Biochemistry 7,* 4011.
Drummond, G. I., and Duncan, L. (1970), *J. Biol. Chem. 245,* 976.
Engelhard, V. H., Esko, J. D., Storm, D. R., and Glaser, M. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73,* 4482.
Esko, J. D., Gilmore, R., and Glaser, M. (1977), *Biochemistry 16,* 1881.
Glaser, M., Ferguson, K. A., and Vagelos, P. R. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 4072.
Goldfine, I. D., Roth, J., and Birnbaumer, L. (1972), *J. Biol. Chem. 247,* 1211.
Higuchi, K. (1970), *J. Cell. Physiol. 75,* 65.
Kahn, T. M. M., and Martell, A. E. (1966), *J. Am. Chem. Soc. 88,* 668.
Kimura, N., and Nagata, N. (1977), *J. Biol. Chem. 252,* 3829.
Kimura, N., Nakane, K., and Nagata, N. (1976), *Biochem. Biophys. Res. Commun. 70,* 1250.
Koshland, D. E., Jr., Nemethy, G., and Filmer, D. (1966), *Biochemistry 5,* 365.
Krishna, G., and Harwood, J. P. (1972), *J. Biol. Chem. 247,* 2253.
Krishna, G., Harwood, J. P., Barber, A. J., and Jamieson, G. A. (1972), *J. Biol. Chem. 247,* 2253.
Leray, F., Chambaut, A., and Hanoune, J. (1972), *Biochem. Biophys. Res. Commun. 48,* 1385.
Londos, C., and Rodbell, M. (1975), *J. Biol. Chem. 250,* 3459.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265.
Monod, J., Wymann, J., and Changeux, J. P. (1965), *J. Mol. Biol. 12,* 88.
Robison, G. A., Butcher, R. W., and Sutherland, E. W. (1968), *Annu. Rev. Biochem. 37,* 149.
Rodbell, M., Birnbaumer, L., Pohl, S. L., and Krans, H. M. J. (1971), *J. Biol. Chem. 246,* 1877.
Rodbell, M., Lin, M. C., Solomon, Y., Londos, C., Harwood, J. P., Martin, B. R., Rendell, M., and Berman, M. (1975), *Adv. Cyclic Nucleotide Res. 5,* 3.
Russell, T. R., Thompson, W. J., Schneider, F. W., and Appleman, M. M. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 1791.
Salomon, Y., Landos, C., and Rodbell, M. (1974) *Anal. Biochem. 58,* 541.

Hormonal Induction of α_{2u}-Globulin Synthesis in Isolated Rat Hepatocytes†

Ching-Ling C. Chen and Philip Feigelson*

ABSTRACT: Hepatocytes freshly prepared with collagenase synthesize α_{2u}-globulin and other hepatic proteins in vitro at approximately the same rate throughout 30 h of incubation. The newly synthesized proteins are efficiently secreted into the medium throughout this period. That the secretion of proteins by hepatocytes is not due to cell leakage is shown by the fact that 30 μM colchicine prevents the appearance of labeled α_{2u}-globulin and other proteins in the medium. Hepatocytes isolated from animals in different endocrine states synthesize α_{2u}-globulin in vitro at rates consistent with the hormonal effects upon its in vivo biosynthesis. In vitro addition of androgens to hepatocytes isolated from castrated male rats evokes an elevation of general protein synthesis in these hepatocytes. Glucocorticoids, added in vitro, specifically induce an elevated rate of α_{2u}-globulin synthesis.

The α_{2u}-globulin was originally found to be in the urine of male rats and absent from the urine of female rats (Roy & Neuhaus, 1966). Both liver extirpation and immunofluorescent studies have shown that α_{2u}-globulin is synthesized in the parenchymal cells of male rat liver, secreted into the serum, and excreted in the urine (Roy & Raber, 1972; Kurtz et al., 1976b). The rate of in vivo hepatic synthesis of α_{2u}-globulin is under multiple endocrine control: androgens, thyroid hormone, pituitary growth hormone, and glucocorticoids each stimulate α_{2u}-globulin synthesis, while estrogens have been shown to be specific anti-inducers in vivo (Sippel et al., 1975; Kurtz et al., 1976a,b). Previous reports from this laboratory have indicated that the regulation of the synthesis of α_{2u}-globulin by glucocorticoids (Sippel et al., 1975), thyroid hormones (Kurtz et al., 1976a), and sex hormones (Kurtz et al., 1976b) occurs via

† From the Institute of Cancer Research and the Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, New York 10032. *Received May 4, 1978.* This work was supported in part by a grant from the National Institutes of Health CA-22376.

0006-2960/78/0417-5308$01.00/0

hormonal modulation of the level of the hepatic mRNA coding for α_{2u}-globulin.

This complex multihormonal control impedes investigation of the role of the individual hormones and clarification of the biochemical processes they influence in the regulation of this specific mRNA in vivo. It is difficult to identify which hormones act directly on the liver, and which hormones are actually modulating the level of other hormones and thus indirectly affecting the biosynthesis of hepatic α_{2u}-globulin. An in vitro hormone-responsive α_{2u}-globulin synthesizing system would provide direct information about this and would enable exploration of the underlying biochemical events. Thus, we have recently developed an isolated rat hepatocyte system to study the hormonal control of α_{2u}-globulin synthesis in vitro.

Hormonal induction by glucocorticoids of hepatic protein synthesis or enzyme activities in isolated hepatocyte suspensions has been demonstrated (Berg et al., 1972). In this laboratory we have recently shown that induction in hepatocytes of tyrosine aminotransferase activity and biosynthesis requires the presence of both dexamethasone and glucagon or N^6,O^2-dibutyryl-cAMP (Ernest et al., 1977). Fibrinogen synthesis in hepatocytes can also be specifically stimulated by cortisol (Crane & Miller, 1977). In this report, we demonstrate the in vitro glucocorticoidal induction of α_{2u}-globulin synthesis and the androgenic control of total hepatic protein synthesis.

Materials and Methods

Sprague-Dawley rats (280–320 g) were used in all experiments. L-[4,5-^{3}H]Leucine (60 Ci/mmol) was obtained from Amersham/Searle Co. Collagenase (type II) was obtained from Worthington Biochemical Co. Media, sera, and other reagents for cell preparation and incubation were purchased from Grand Island Biological Co. Hormones were obtained from Sigma Chemical Co. Hepes[1] [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] was purchased from Calbiochem.

Cell Preparation and Incubation. Rat liver cells were prepared by a modification of the "two-step procedure" described by Seglen involving the removal of Ca^{2+} from the liver followed by perfusion with collagenase (Seglen, 1976). After digestion with collagenase, the liver was immediately perfused with 50–100 mL of calcium- and magnesium-free Hanks balanced salt solution containing 0.2% dialyzed, fatty acid free bovine serum albumin to remove Ca^{2+} ion and collagenase. Cells were washed several times with the above solution, and damaged and nonparenchymal cells were removed from the supernatant following centrifugation at 50g for 2 min. Cells were counted in a hemacytometer and their viability was determined by exclusion of trypan blue dye. The yield of cells was $7–9 \times 10^8$ cells per/liver and their viability was 90–95%.

In a typical experiment, 15×10^6 viable cells were suspended in 10 mL of Joklik modified minimum essential medium containing 25 mM Hepes (pH 7.5) and 10% fetal calf serum. The cell suspensions were incubated at 37 °C in a 50-mL polypropylene Erlenmayer flask with gentle rotational shaking (80 rpm) under an atmosphere of 95% O_2 and 5% CO_2 (Ernest et al., 1977). Hormones and labeled precursors were added as indicated.

Precursor Incorporation into Protein. [^{3}H]Leucine (5 μCi/μL) was added to the cell suspensions at the indicated times of incubation. The rate of total protein synthesis was measured by withdrawing 25-μL samples from cell suspensions at the indicated times and spotting them on 2.3-cm Whatman 3MM filter paper discs which were then immersed in cold 10% trichloroacetic acid containing 10 mM leucine. The filters were processed as described by Mans & Novelli (1960). The rate of secretion of hepatic proteins into the medium was measured by separation of media from cells at indicated times by centrifugation at 50g for 3 min. Twenty-five microliters of in vitro labeled media was spotted on Whatman filter discs and processed as described above. The cells were washed with phosphate-buffered saline (pH 7.2). Cells and media were then frozen in liquid nitrogen for later use. For pulse-labeling studies, cells were incubated for varied time in the medium as described above. At the indicated times, the viable cells were collected by centrifugation, washed, and resuspended in leucine-free Joklik modified MEM containing 50 μCi per mL of [^{3}H]leucine as the sole source of leucine. The cells were harvested after 30–60 min of incubation in this medium and frozen in liquid nitrogen.

Preparation of Cell Cytosols. Frozen cells were resuspended in 0.25 M sucrose, 5 mM magnesium acetate, 25 mM sodium chloride, and 25 mM Hepes (pH 7.5). The cells were disrupted by sonication, brought to 1% Triton X-100 and sodium deoxycholate and vigorously mixed for 30 s. Cytosols were prepared from the lysed cells by centrifugation at 105 000g for 60 min.

Immunoprecipitation and Isolation of ^{3}H-Labeled α_{2u}-Globulin. The immunoprecipitation reaction mixture contained 10 mM sodium phosphate (pH 7.2), 2% Triton X-100, 10 mM leucine, 250–300 μL of anti-α_{2u}-globulin IgG, a suitable amount of purified α_{2u}-globulin as carrier (total α_{2u}-globulin in the mixture was 10 μg) and in vitro labeled α_{2u}-globulin from liver cells and/or media. Following incubation for 1 h at 25 °C and 6–8 h at 0–4 °C, the immunoprecipitate was collected, washed, and solubilized as described by Sippel et al. (1975). The dissolved immunoprecipitates were subjected to $NaDodSO_4$–polyacrylamide gel electrophoresis. After electrophoresis, the gels were frozen and sectioned into 2-mm slices. Each gel slice was incubated in Econofluor containing 3% Protosol (New England Nuclear Co.) for 24 h at 37 °C and counted with a Packard scintillation counter. Radioactivity incorporated into α_{2u}-globulin was expressed as the sum of the counts per min after correction for the background in the α_{2u}-globulin peak of the $NaDodSO_4$–polyacrylamide gels.

Results

The Synthesis and Secretion of Hepatic Proteins by the Cells. The isolated hepatocyte suspensions synthesized and secreted hepatic protein for over 30 h. The viability of freshly prepared cells measured by trypan blue dye exclusion was always initially 90–95%. Cellular viability decreased at a rate of 1–2% per h of incubation. Isolated liver cells efficiently incorporated labeled precursors into hepatic protein (Figure 1). Incorporation of [^{3}H]leucine into total hepatic proteins continued at an approximately linear rate for over 30 h of incubation. The newly synthesized protein was secreted into the medium where it accumulated and at 12 h represented approximately 40–50% of the total newly synthesized protein. In order to determine that the appearance of labeled proteins in the medium is due to active secretion and not to cell leakage, cell suspensions were also incubated with 30 μM colchicine, a drug known to inhibit protein secretion by binding to microtubules which are necessary for protein transport out of the cell. As shown in Figure 1, 30 μM colchicine largely prevented the appearance of ^{3}H-labeled protein in the medium; most of the newly synthesized protein was intracellular.

[1] Abbreviations used: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid; $NaDodSO_4$, sodium dodecyl sulfate; MEM, minimum essential medium.

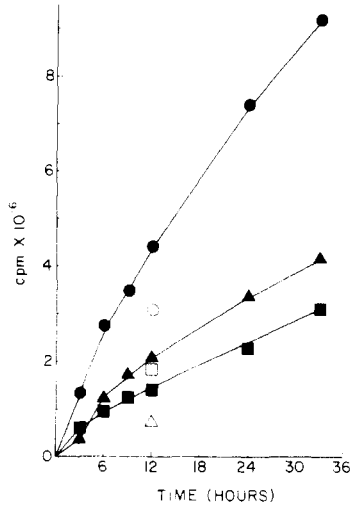

FIGURE 1: Time course of synthesis and secretion of hepatic protein by rat hepatocytes. The 15 × 10^6 viable cells from an adult male rat were incubated in 10 mL of Joklik modified minimum essential medium containing 10% fetal calf serum, and 125 μCi of [^{3}H]leucine. As described in Materials and Methods, the amount of total hepatic protein synthesis was determined by the amount of [^{3}H]leucine incorporated into protein. The rate of incorporation of [^{3}H]leucine into protein was measured by precipitating an aliquot of cell suspension with 10% trichloroacetic acid on paper discs. At time intervals an aliquot of cell suspensions was removed and centrifuged at 50 g for 2 min to separate cells and medium. Liver cytosol (S-100) was prepared from the supernatant of high speed (105 000g) ultracentrifugation of sonicated cells. At each time point, total protein (●), secreted protein (▲), and cytosol protein (■) were measured. The effect of colchicine on secretion of hepatic proteins by these cells was determined by incubating the cells with 30 μM colchicine in the medium as described above. Total (○), secreted (△), and cytosol (□) proteins were measured at each time point.

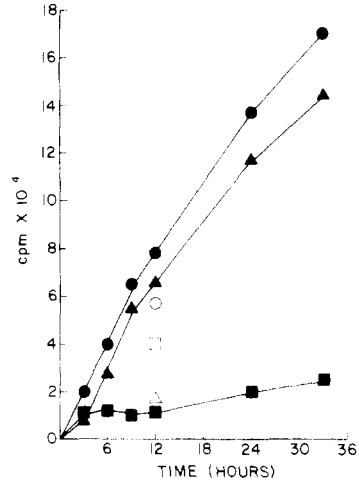

FIGURE 2: Time course of synthesis and secretion of α_{2u}-globulin by hepatocytes. The 15 × 10^6 viable cells from adult male rat were incubated as described previously. The amount of newly synthesized α_{2u}-globulin was isolated and quantitated by immunoprecipitation with anti-α_{2u}-globulin followed by NaDodSO$_4$-polyacrylamide gel electrophoresis; total α_{2u}-globulin synthesized in liver (●), α_{2u}-globulin secreted into the medium (▲), α_{2u}-globulin remaining in cytosol (■). Radioactivity incorporated in α_{2u}-globulin was expressed as the sum of the counts per min in the immunoprecipitate peak after correction for the background. Open symbols depict the total (○), secreted (△), and cytosolic (□) α_{2u}-globulin synthesis from cells incubated with 30 μM colchicine.

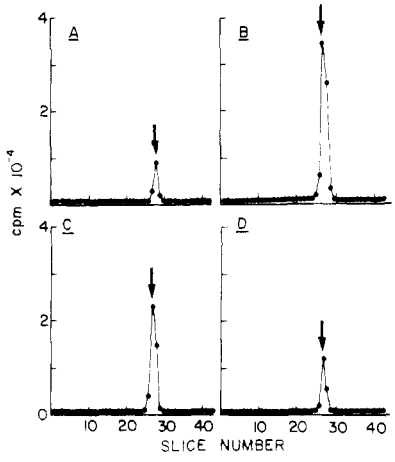

FIGURE 3: The NaDodSO$_4$-polyacrylamide gel electrophoresis pattern of α_{2u}-globulin synthesized by isolated hepatocytes. The 15 × 10^6 viable cells from an adult male rat were incubated in medium containing 12.5 μCi/mL of [^{3}H]leucine as described previously. After 12 h of incubation, the ^{3}H-labeled protein from cytosol and medium were immunoprecipitated with anti-α_{2u}-globulin followed by NaDodSO$_4$ electrophoresis. (A) Cytosol; (B) medium; (C) cytosol from cells treated with 30 μM colchicine; (D) medium from cells treated with colchicine.

Immunoprecipitation of the in vitro synthesized proteins with monospecific anti-α_{2u}-globulin followed by NaDodSO$_4$-polyacrylamide gel electrophoresis of the dissolved immunoprecipitate enabled monitoring of the synthesis and secretion of α_{2u}-globulin (Figure 2). Total α_{2u}-globulin was synthesized essentially linearly throughout 33 h of incubation, and, as in vivo, 80–90% of the newly synthesized α_{2u}-globulin was secreted into the medium. Initially the newly synthesized α_{2u}-globulin accumulated in the hepatocyte cytosol and reached a steady-state level. The labeled protein was secreted into the medium where its level increased throughout the incubation period. The secretion of this protein could be prevented by the addition of 30 μM colchicine, whereupon 70–80% of the newly synthesized α_{2u}-globulin accumulated within the cells (Figures 2 and 3).

The NaDodSO$_4$-polyacrylamide gel electrophoresis patterns of anti-α_{2u}-globulin immunoprecipitates of [^{3}H]leucine-labeled proteins of cytosol and of medium are shown in Figure 3. They indicate in each instance a single peak which migrates at the 20 000 dalton position of authentic α_{2u}-globulin. As described above, most of the [^{3}H]leucine-labeled α_{2u}-globulin normally secreted into the medium and that secretion can be prevented by colchicine.

Synthesis of α_{2u}-globulin by these hepatocytes was prevented when inhibitors of protein synthesis (cycloheximide) or of RNA synthesis (actinomycin D) were added to the cell suspensions (Figure 4). Actinomycin D decreased total protein synthesis by 30% during 12 h of incubation, and by 95% during 24 h of incubation. However, the rate of α_{2u}-globulin synthesis was reduced less by actinomycin D: 15–20% decrease by 12 h and 75% decrease by 24 h of incubation. Although only tentative inferences may be drawn from such inhibitor experiments these results suggest that the mRNA for α_{2u}-globulin may be more stable than those of other hepatic mRNAs, i.e., the half-life of α_{2u}-globulin mRNA may be longer than that of mRNA for the average hepatic protein. The syntheses of both total hepatic proteins and α_{2u}-globulin were inhibited 90–95% by cycloheximide at 12 h, and almost completely abolished by 24 h of incubation.

Effect of the in Vivo Endocrine State on the in Vitro Synthesis of α_{2u}-Globulin by Hepatocytes. Hepatocytes isolated from an adult male rat synthesized α_{2u}-globulin at a rate corresponding to that which occurs in vivo, i.e., 1–2% of total hepatic protein synthesis. As mentioned before, α_{2u}-globulin is androgen inducible and estrogen repressible (Kurtz et al.,

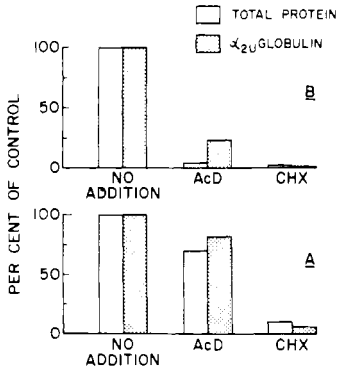

FIGURE 4: Effects of inhibitors on synthesis of total hepatic protein and α_{2u}-globulin in hepatocytes. The 15 × 10^6 viable cells from an adult male rat were incubated as described previously. Total hepatic protein and α_{2u}-globulin synthesis were determined as described in Materials and Methods. (A) [^{3}H]Leucine incorporation at 0–12 h of incubation; (B) incorporation at 12–24 h. Inhibitors were added at zero time and were present throughout the incubation. In B, the cells were incubated with inhibitors without [^{3}H]leucine for 0–12 h. At 12 h fresh media containing inhibitors and 125 μCi of [^{3}H]leucine were added. The cells were harvested at 24 h. The concentration for actinomycin D (AcD) is 2.5 μM, and for cycloheximide (CHX) is 50 μM.

TABLE I: Effect of Endocrine State of Animals on the Synthesis of α_{2u}-Globulin by Hepatocytes.[a]

animal condition	hepatocyte	in vivo
intact male	100	100
intact female	0	0
ovariectomized female, 14 days	0	0
castrated male, 5 days	47	
castrated male, 14 days	33	15
estradiol treated male,[b] 2 days	52	65
adrenalectomized male, 5 days	33	25
adrenalectomized male, 14 days	21	20

[a] All values are percent of synthesis for intact adult males. In isolated hepatocyte systems, intact male hepatocytes synthesize α_{2u}-globulin which represents 1.5% of total protein synthesis during 12 h of incubation. In vivo α_{2u}-globulin synthesis in intact males is 1.0% of total hepatic protein synthesis following pulse incorporation of [^{3}H]leucine for 12 min. The synthesis of α_{2u}-globulin was measured by immunoprecipitation followed by $NaDodSO_4$-polyacrylamide gel electrophoresis as described in Materials and Methods. [b] Male rats were injected on successive days subcutaneously with estradiol (0.5 mg/(kg day)) in an emulsion containing (v/v/v) 89.6% 0.1 M sodium phosphate (pH 7.2), 10% propylene glycol, 0.4% Tween 80.

1976b). Isolated hepatocytes derived from intact female or spayed female rats produced no detectable α_{2u}-globulin (Table I). Castration or estrogen administration to male rats reduced α_{2u}-globulin synthesis equivalently in vivo and by their hepatocytes in vitro. Likewise, adrenalectomy of adult male rats results in a 60–80% reduction in α_{2u}-globulin synthesis, both in vivo and by their hepatocytes in vitro. Therefore, liver cells prepared from rats in different endocrine states synthesize α_{2u}-globulin in vitro, to an extent consistent with the hormonal effect upon its in vivo biosynthesis (Table I).

In Vitro Hormonal Control of α_{2u}-Globulin Synthesis in Isolated Hepatocytes. Addition of 5α-dihydrotestosterone to medium bathing hepatocytes isolated from 14-day-castrated male rats resulted in a twofold increase in [^{3}H]leucine incorporation into α_{2u}-globulin after 24 h of incubation (Figure 5). However, this apparent induction was accompanied by a comparable increase in [^{3}H]leucine incorporation into total hepatic proteins. The androgen effect obtainable under these

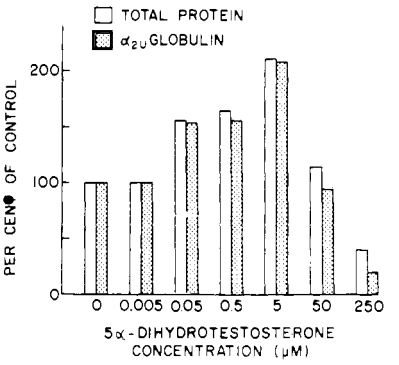

FIGURE 5: Induction of total hepatic protein and α_{2u}-globulin by androgen. The 15 × 10^6 viable cells isolated from a 14-day-castrated male rat were incubated with various concentrations of 5α-dihydrotestosterone for 24 h. The cells were then placed in fresh leucine-free medium containing the same androgen level as before and pulse labeled with [^{3}H]-leucine for 60 min as described in Materials and Methods. Total protein and α_{2u}-globulin synthesis were measured as described before.

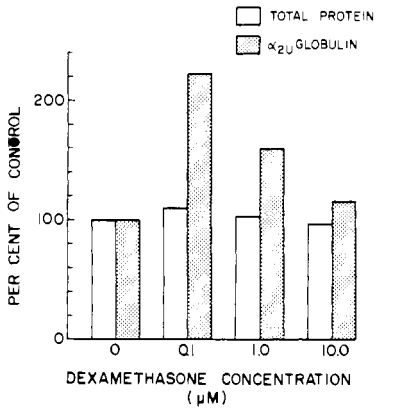

FIGURE 6: Induction of α_{2u}-globulin synthesis by dexamethasone. The 15 × 10^6 viable cells isolated from a 14-day-castrated male rat were incubated with the indicated concentrations of dexamethasone for 9 h. The cells were then placed in fresh leucine-free Joklik modified MEM also containing the indicated level of dexamethasone and pulse labeled with [^{3}H]leucine for 30 min as described in Materials and Methods.

conditions was thus a general effect and not specific for α_{2u}-globulin synthesis. The optimal concentration for maximal androgenic effect is 5 μM. Addition of testosterone instead of 5α-dihydrotestosterone to these cells showed the same general stimulation phenomenon except that the optimal concentration of testosterone required for this effect is much lower, 50 nM. Similar results were obtained if the fetal calf serum used in the medium had been pretreated with charcoal-dextran to remove endogenous steroid hormones (Sato, 1974).

In contrast to the nonspecific androgen effect, glucocorticoids exert a selective action on the in vitro synthesis of α_{2u}-globulin. In vitro addition of dexamethasone to hepatocytes from castrated male rats resulted in a twofold increase of [^{3}H]leucine incorporation into α_{2u}-globulin, while total hepatic protein synthesis remained unaffected by this steroid (Figure 6). Hepatocytes isolated from castrated rats synthesized α_{2u}-globulin at a level which represented 0.4–0.5% of total hepatic protein synthesis; in vitro addition of dexamethasone to these cell suspensions resulted in a rise of α_{2u}-globulin to 0.8–1.0% of the total hepatic proteins synthesized (Table II). This indicates that the induction of dexamethasone is specific for α_{2u}-globulin. The dexamethasone dose-response curve for induction of this protein synthesis (Figure 6) indicated an optimal concentration of 0.1 μM, which approached physio-

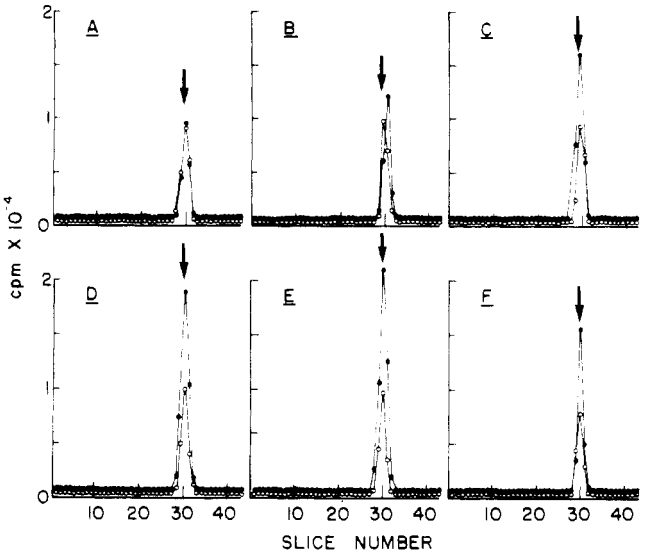

FIGURE 7: Time course of induction of α_{2u}-globulin synthesis by dexamethasone. The 15 × 10^6 viable cells isolated from the liver of a 14-day-castrated male rat were incubated with (●) or without (○) 0.1 μM dexamethasone. At indicated times, the cells were placed in fresh leucine-free medium with or without dexamethasone and pulse labeled with [^{3}H]leucine (50 μCi/mL) for 30 min: (A) 2, (B) 4, (C) 6, (D) 8, (E) 10, (F) 12 h.

TABLE II: Induction of α_{2u}-Globulin Synthesis by Glucocorticoids in Hepatocytes in Vitro.[a]

endocrine state	α_{2u}-globulin as % of total protein synthesis	% control
intact male	1.50	100
intact male + dexamethasone (0.1 μM)	1.45	97
castrated male[b]	0.50	33
castrated male + dexamethasone (0.1 μM)	1.00	67
adrenalectomized male (Adx)[c]	0.50	33
Adx + Dex (10 μM)	0.76	51
estradiol-treated male[d]	0.50	33
estradiol-treated male + Dex (0.1 μM)	0.68	46

[a] The 15 × 10^6 viable hepatocytes were incubated in Joklik modified MEM as described in Figure 1 for 10 h. The cells were then replaced in fresh leucine-free Joklik-modified MEM and pulse labeled with [^{3}H]leucine for 60 min as described in Materials and Methods. The newly synthesized α_{2u}-globulin was measured by immunoprecipitation followed by $NaDodSO_4$-polyacrylamide gel electrophoresis as described in Materials and Methods. [b] Fourteen days following castration. [c] Five days after adrenalectomy. [d] Male rats were injected subcutaneously with 17β-estradiol (0.5 mg/(kg day)) for 5 days as described in the legend to Table I.

logical levels (King & Mainwaring, 1974). This is also consistent with our earlier studies for induction of the intracellular enzyme tyrosine aminotransferase by glucocorticoids in isolated hepatocytes (Ernest et al., 1977). The time course for the increase of [^{3}H]leucine incorporation into α_{2u}-globulin by dexamethasone is illustrated in Figure 7. Following a 2-h lag, increased α_{2u}-globulin synthesis started to appear at 3–4 h, reached a maximum at 10 h, and persisted at 12 h under treatment with dexamethasone. Again a 1.7–2.2-fold increase of α_{2u}-globulin synthesis was observed after 9–10 h of incubation with this steroid.

The glucocorticoid induction effect was also studied in the hepatocytes derived from animals in various endocrine states (Table II). Addition of dexamethasone to hepatocytes isolated from intact adult males does not induce α_{2u}-globulin synthesis above its control level. However, if the intracellular level of α_{2u}-globulin synthesis is depressed by pretreatment with 17β-estradiol in vivo, the in vitro synthesis of this protein can reproducibly be elevated by glucocorticoids.

Our previous results (Sippel et al., 1975) have shown that adrenalectomy reduced α_{2u}-globulin synthesis in vivo and administration of glucocorticoids restored the synthesis of this protein. This in vitro study shows a similar phenomenon. Adrenalectomy causes a reduction of α_{2u}-globulin synthesis in isolated hepatocytes (Tables I and II). Addition of dexamethasone to the hepatocytes isolated from these male rats results in a 50% increase in the synthesis of this protein. The induction of α_{2u}-globulin synthesis is smaller in adrenalectomized livers than observed in livers of castrated males.

Discussion

Freshly prepared hepatocytes synthesize and secrete hepatic proteins linearly for 36 h. This affords the opportunity for in vitro study of relatively long term processes, such as the action of steroid hormones on gene expression. Immunoprecipitation of the in vitro labeled hepatic protein with anti-α_{2u}-globulin followed by $NaDodSO_4$-polyacrylamide gel electrophoresis indicated that α_{2u}-globulin is efficiently synthesized and actively secreted into the medium by these liver cells. Hepatocytes isolated from rats in different endocrine states synthesize α_{2u}-globulin in vitro to an extent consistent with the hormonal effect upon its in vivo biosynthesis. This is thus an in vitro system which behaves similarly to liver in vivo and enables the study of the complicated hormonal control of α_{2u}-globulin synthesis.

We have previously reported that rat liver cells isolated with collagenase by a modified method of Seglen showed hormonal responsiveness in that tyrosine aminotransferase activity and its biosynthesis were induced in the presence of both dexamethasone and glucagon or dibutyryl-cAMP (Ernest et al., 1977). In the present study, we have found that α_{2u}-globulin synthesis can be selectively induced by dexamethasone in hepatocytes isolated from male rats having a low rate of α_{2u}-globulin synthesis in vivo, i.e., adrenalectomized, castrated, or estrogen-treated males.

Castration of male rats has been shown to reduce the synthesis of α_{2u}-globulin and the level of its mRNA. Administration of androgen resulted in a reinduction in the synthesis of this protein and elevation of its mRNA in vivo (Kurtz et al., 1976b). In our in vitro hepatocyte system, we found that the rate of synthesis of α_{2u}-globulin is also depressed in the hepatocytes isolated from castrated males. However, addition of androgens to these cell suspensions resulted in a general increase of hepatic protein synthesis as well as α_{2u}-globulin synthesis. The androgen effect is therefore not selective for α_{2u}-globulin. This is quite different from the androgenic effect in vivo. The androgenic effect upon α_{2u}-globulin synthesis and its mRNA level in vivo may reflect intricate physiological events rather than exclusive androgenic effects upon the liver.

In contrast to androgen, glucocorticoids exert a selective action on the synthesis of hepatic α_{2u}-globulin. In vitro addition of dexamethasone to the hepatocytes isolated from castrated, estrogen-treated, or adrenalectomized males results in an induction of α_{2u}-globulin synthesis, while total hepatic protein synthesis was not affected by this steroid. It suggests that glucocorticoids exert a direct control on the synthesis of this hepatic protein in liver. In this study, we showed that glucocorticoids cannot induce α_{2u}-globulin synthesis in hepatocytes

derived from intact males. However, this steroid can reinduce this protein synthesis in the hepatocytes isolated from either androgen or glucocorticoid deficient males. This is in good agreement with previous results which indicate that the rate of synthesis of α_{2u}-globulin in intact males is at the fully induced level. The administration of any hormone(s) to intact males does not induce further α_{2u}-globulin synthesis in vivo. Only when the rate of α_{2u}-globulin synthesis is reduced by adrenalectomy, castration, or thyroidectomy can hormones reinstate the full biosynthetic rate. Whether the specific glucocorticoidal induction of α_{2u}-globulin synthesis in hepatocytes is via transcriptional, posttranscriptional, or translational control is under investigation in this laboratory.

References

Berg, T. Boman, D., & Seglen, P. O. (1972) *Exp. Cell. Res. 72,* 571-574.

Crane, L. J., & Miller, D. L. (1977) *J. Cell Biol. 72,* 11-25.

Ernest, M. J., Chen, C.-L., & Feigelson, P. (1977) *J. Biol. Chem. 252,* 6783-6791.

King, R. J. B., & Mainwaring, W. I. P. (1974) *Steroid-Cell Interactions,* University Park Press, Baltimore, Md.

Kurtz, D. T., Sippel, A. E., & Feigelson, P. (1976a) *Biochemistry 15,* 1031-1036.

Kurtz, D. T., Sippel, A. E., Ansah-Yiadom, R., & Feigelson, P. (1976b) *J. Biol. Chem. 251,* 3594-3598.

Mans, R. J., & Novelli, G. D. (1960) *Biochem. Biophys. Res. Commun. 3,* 540-543.

Roy, A. K., & Neuhaus, O. W. (1966) *Proc. Soc. Exp. Biol. Med. 121,* 894-899.

Roy, A. K., & Raber, D. L. (1972) *J. Histochem. Cytochem. 20,* 89-96.

Sato, G. (1974) *Methods Enzymol. 32B,* 557-561.

Selgen, P. O. (1976) in *Methods in Cell Biology* (Prescott, D. M., Ed.) Vol. 13, pp 29-83, Academic Press, New York, N.Y.

Sippel, A. E., Feigelson, P., & Roy, A. K. (1975) *Biochemistry 14,* 825-829.