CONTROL OF GROWTH HORMONE PRODUCTION BY A CLONAL STRAIN OF RAT PITUITARY CELLS

Stimulation by Hydrocortisone

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ABSTRACT

Addition of hydrocortisone to the medium of a clonal strain of rat pituitary cells (GH_3) stimulated the rate of production of growth hormone. The stimulation had a lag period of about 24 hr, reached a maximum at 70–100 hr, and was observed at a hydrocortisone concentration as low as 5×10^{-8} M. Cells maximally stimulated with 3×10^{-6} M hydrocortisone produced 50–160 μg growth hormone/mg cell protein/24 hr. These rates were four to eight times those observed in control cells. At maximum stimulation, intracellular levels of growth hormone in both stimulated and control cells were equal to the amount secreted into the medium in about 15 min. Removal of hydrocortisone from the medium of GH_3 cells caused a return of the rate of growth hormone production to that in control cells. Addition of hydrocortisone to the medium of cells growing exponentially with a population-doubling time of 60 hr caused both an increase in the doubling time to 90 hr and a stimulation of growth hormone production. Cycloheximide (3.6×10^{-5} M) and puromycin (3.7×10^{-4} M) suppressed incorporation of labeled amino acids into protein by 93 and 98%, respectively, and suppressed growth hormone production by stimulated and control cells by at least 94%.

INTRODUCTION

Growth hormone is a single polypeptide chain with a molecular weight of about 20,000 (1). It is, therefore, reasonable to suppose that its synthesis is similar to that of other mammalian cell proteins. Studies to date of the hormonal control of the production of organ-specific proteins by cells in culture have centered on enzymes which carry out their function intracellularly. Notable among such studies are those of Tomkins and coworkers (2) and Reel and Kenney (3) on the induction by hydrocortisone of tryrosine aminotransferase in cultured hepatoma cells, and by Amos and coworkers (4, 5) and Moscona and his colleagues (6) on the induction by hydrocortisone of glutamine synthetase in freshly explanted chick embryo retinal cells.

These studies have revealed that the level of an enzyme in cells in culture represents the resultant of synthesis and degradation of enzyme molecules. In constructing a model for the effect of a hormone on the intracellular levels of an enzyme, one must, therefore, consider the possibility of effects not only on transcription and translation, but on

turnover of enzyme molecules as well. Furthermore, the fact that this turnover exists makes it difficult to determine experimentally whether an observed increase in enzyme activity reflects an increased rate of synthesis of the enzyme, a decrease in the rate of its degradation, or a combination of both of these effects.

Our studies on the control of production of growth hormone, a nonenzymic, organ-specific protein, should complement the studies of the control of enzyme production cited above. Furthermore, although the pituitary gland in vivo stores large amounts of growth hormone, we have found that the cultured rat pituitary cells (GH_3) used in the present investigations secrete growth hormone quite rapidly after it is synthesized. Thus, to the extent that the secretion of growth hormone spares it from turnover within the cells which produce it, both conceptual and experimental complications arising from intracellular degradation should be largely circumvented.

Kohler et al. (7) have reported that hydrocortisone stimulates the production of growth hormone by primary mixed cultures of monkey pituitary glands. We have further investigated this aspect of the hormonal control of growth hormone production in GH_3 cells.[1]

MATERIALS AND METHODS

Materials

Viokase was purchased from the VioBin Corporation, Monticello, Ill. Hydrocortisone hemisuccinate was obtained from the Upjohn Company, Kalamazoo, Mich. Stock solutions of hydrocortisone were made in 0.15 M NaCl at a concentration of 9×10^{-5} M, and stored at 4°C for no more than 1 wk. A mixture of ^{14}C-labeled amino acids (^{14}C-reconstituted protein hydrolysate, 103–330 mc/mmole a.a., 100 μc/ml) was obtained from Schwarz BioResearch, Inc., Orangeburg, N. Y. Cycloheximide and puromycin dihydrochloride were purchased from Nutritional Biochemicals Corporation, Cleveland.

Methods of Culture and Growth Hormone Assay

The origin of rat pituitary tumor cells and methods of culture of the GH_3 strain have been described in detail previously (9). Experiments were performed in plastic tissue culture dishes (50 × 15 mm, Falcon Plastics, Los Angeles) containing 3 ml (unless otherwise noted) of Ham's F 10 medium (10) supplemented with 15% horse serum and 2.5% fetal calf serum (complete F 10). The dishes were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Medium was changed every 2 or 3 days before the beginning of an experiment.

Some experiments were also carried out in media chemically better defined than complete F 10. However, in either F 10 supplemented with dialyzed serum or F 10 containing no serum, the basal rate of GH[2] production by GH_3 cells dropped precipitously within a few days, and the cells exhibited cytopathological changes. Thus, although a substantial stimulation of GH production by hydrocortisone could be observed under both of these conditions, we decided to use complete F 10 medium for all studies reported here.

At the time these experiments were carried out, the GH_3 cells were shown to be free of pleuropneumonia-like organisms by Dr. Iolanda K. Low of the Department of Bacteriology and Immunology, Harvard Medical School.

GH was assayed immunologically in culture medium or in cell homogenates by the method of complement fixation (11). The lower limits of detection of GH by this method are 0.1–0.025 μg/ml tissue culture medium. The reproducibility of the method as performed in these experiments is ± 20%.

Experiments designed to assess cell growth and function were performed on groups of identical dishes established from a homogeneous suspension of cells which had been dispersed with 0.1% Viokase. Dishes from which medium was saved for GH assay were washed twice with isotonic saline and stored at −20°C until cell protein was determined by the method of Lowry et al. (12).[3]

Specification of the Growth State of GH_3 Cells

When dispersed with Viokase and plated sparsely in complete F 10 medium, GH_3 cells, after a lag period of 1–2 days, grow exponentially with a population-doubling time[4] of about 60 hr. The cells

[1] A preliminary report of these findings has been made (8).

[2] Abbreviations used: GH, growth hormone; HC, hydrocortisone hemisuccinate; TCA, trichloroacetic acid; SDS, sodium dodecyl sulfate; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-2-(5-phenyloxazolyl)-benzene.

[3] Protein determinations and cell counts in a hemocytometer, performed on aliquots of a suspension of cells which had been in early stationary phase, indicated that 1.0 mg of cell protein corresponds to about 6×10^6 cells.

[4] The term "population-doubling time" as used in this report refers to the time required for the cell protein per dish to double.

eventually reach a state in which the cell protein stops increasing exponentially and either levels off or increases at a greatly reduced rate. We refer to this state of cell growth as *early stationary phase.*

Incorporation of ^{14}C-Labeled Amino Acids into Protein

Approximately 2.6 × 10^6 cpm (2 μc) ^{14}C-reconstituted protein hydrolysate was added to the medium of each dish. At the end of the incubation period (70 min), the medium was removed and frozen for later determination of TCA-insoluble counts. The dishes were washed twice with ice-cold Tris-saline buffer (0.1 M NaCl, 0.02 M Tris, pH 8.5), and 1.0 ml of 1% SDS buffer (1% SDS, 0.005 M EDTA, 0.1 M NaCl, 1% mercaptoethanol, 0.02 M Tris, pH 8.5) was added. After 10–15 min of incubation at room temperature, the SDS suspensions were transferred to glass tubes. Each dish was washed with an additional 1.0 ml of SDS buffer, which was pooled with the original suspension. The tubes were then stoppered and placed in boiling water for 15 min so as to solubilize the suspension. Duplicate 0.8-ml aliquots were added to vials containing 0.15 mg of carrier bovine serum albumin. TCA (50%) was added to a final concentration of 6%, and the vials were put on ice for 15 min. The contents of the vials were then filtered through Schleicher and Schuell filters which has been pretreated with 5% TCA. The filters were washed successively with cold 5% TCA, a 1:1 mixture of ethanol and ether, and TCA. They were then air-dried overnight, and counted in 10 ml of toluene scintillation fluid (4 g of PPO and 0.05 g of POPOP in 1 liter of toluene) in a Beckman Model LS 150 scintillation counter.

The number of counts in the medium was estimated by placing 20 μl directly onto filters, drying, and counting as described above. Such measurements performed on medium at the beginning and end of the incubation period indicated that a negligible fraction of the added counts had disappeared from the medium during this interval.

TCA precipitation, filtration, and counting of aliquots of the medium removed at the end of the 70-min pulse revealed that less than 4% of the counts incorporated into TCA-insoluble material during this time had been released into the medium. Hence the intracellular TCA-insoluble material represents to a good approximation the total incorporation of labeled amino acids into TCA-insoluble material during this interval.

RESULTS

Changes in the GH_3 Line Since Its Establishment

The GH_3 cell strain was established in 1965. Results obtained with both a radioimmunoassay method and a complement fixation assay demonstrated that these cells secreted into the medium a material immunologically indistinguishable from rat growth hormone. During the first 25 months following the strain's initiation in culture, neither prolongation of the generation time nor diminution of the rate of hormone secretion was observed (9), indicating that the cells of this strain were not undergoing "senescence" during this time.

In the present experiments, performed approximately 3½ yr after establishment of the GH_3 line, we have observed a population-doubling time for these cells in complete F 10 medium of about 60 hr (see Fig. 4 below), compared to the value of about 40 hr originally observed. The rate of GH production in early stationary phase is now about three to five times greater than that originally observed (15–30 compared to 4–6 μg GH/mg cell protein/24 hr). Thus, the ability of these cells to carry out a specialized function has increased during prolonged serial propagation in culture.

Stability of Growth Hormone

An experiment was performed to determine the stability of rat GH under the conditions of incubation used in the present studies. Medium containing added pure rat GH was placed in dishes which

TABLE I

Stability of Growth Hormone

Conditions	Growth hormone	Recovery
	μg/ml	%
Stock solution	17	—
GH_3 cells alone	2.7 ± 0.9	—
Stock solution incubated without cells	17	100
Stock solution incubated with GH_3 cells	18 ± 1.0	91

A stock solution was made by adding rat GH to complete F 10 medium. 3 ml aliquots of the stock solution were then added to a cell-free dish and to two replicate dishes containing GH_3 cells, and the remaining solution was frozen. Two replicate dishes of GH_3 cells which received only complete F 10 medium served as controls to determine the amount of growth hormone produced by the GH_3 cells. After a 72-hr incubation, the medium was removed from each dish and frozen. The collected media and the stock solution were then assayed for GH. The ranges of the results in duplicate dishes are shown.

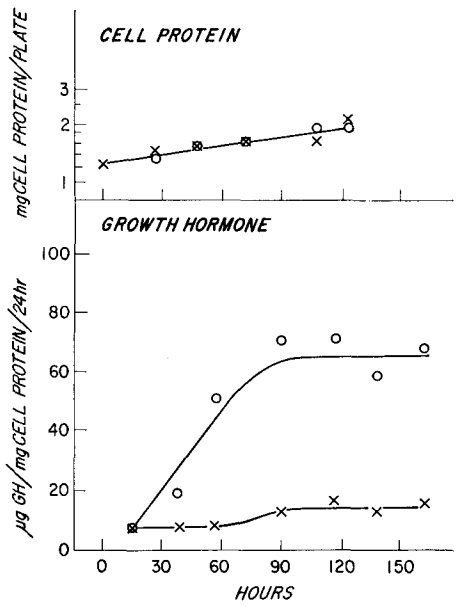

FIGURE 1 Stimulation of GH production by HC. Replicate dishes containing cells in early stationary phase were used. At zero time, fresh medium containing either HC (3×10^{-6} M) or lacking HC was added to each dish. Every 24 hr the medium was collected from an HC-treated and a control dish and frozen for GH assay. These dishes were washed twice with isotonic saline and frozen for the determination of cell protein. The medium on all remaining dishes was then changed, with medium containing HC being added to the appropriate dishes. (×—× = −HC; ○—○ = +HC).

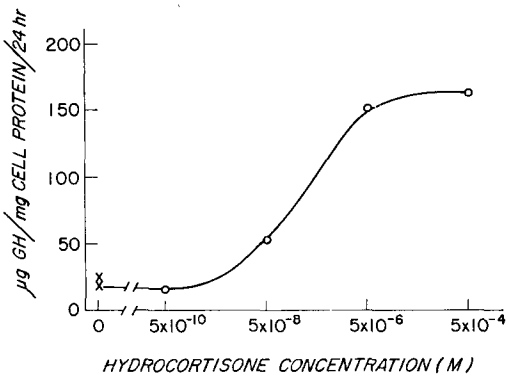

FIGURE 2 Effects of various concentrations of HC on GH production. Conditions were the same as described in Fig. 1, except that medium containing HC at final concentrations from 5×10^{-10} M to 5×10^{-4} M was added to experimental dishes, and medium lacking HC was added to duplicate control dishes. Rates of GH production in the interval 96–121 hr are shown. (× = −HC; ○ = +HC).

TABLE II

Duration of Stimulation by Hydrocortisone of Growth Hormone Production

Collection period	Growth hormone production		Ratio
	−HC	+HC	
hr after addition of HC	*μg GH/mg cell protein/24 hr*		*+HC/−HC*
0–71	4.9	35	7.1
71–120	17	81	4.8
120–163	12	101	8.4
163–214	21	103	4.9
214–256	32	141	4.4

Cells were plated sparsely in replicate dishes 3 days before beginning the experiment. Cell protein increased exponentially until about 165 hr (−HC) or 210 hr (+HC), at which time the cells entered early stationary phase. Conditions of the experiment were as described in Fig. 1, except the medium was collected and changed every 2 days.

contained either no cells or GH_3 cells. After a 3-day incubation period, the medium was removed and assayed for GH. The results (Table I) show that 100% of the GH originally added could be recovered from the cell-free dishes and 91% from those containing GH_3 cells. Since medium was changed at least every 2 days in the experiments reported below, we conclude that in those experiments virtually all of the GH secreted into the medium during an incubation period was still present when the medium was collected and assayed.

Stimulation of Growth Hormone Production by Hydrocortisone

Medium containing hydrocortisone (3×10^{-6} M) was added to GH_3 cells in early stationary phase (Fig. 1).[5] During the first 24 hr, no stimulation of the rate of production of GH was observed. Thereafter, GH production in hydrocortisone-

[5]It should be emphasized that since the rate of GH production reported in these studies is, in actuality, a measurement of the amount of GH which accumulated in the medium during a given period of time, it represents the *average* rate of appearance during this period. It should also be noted that the terms "rate of production of GH" and "rate of appearance of GH in medium" are used interchangeably in reporting experimental results. The justification for this terminology is presented in the Discussion.

treated cells increased compared to that in control cells. At about 90 hr, GH production reached a maximum rate about five times that in control cells.

Effect of Various Concentrations of Hydrocortisone on Growth Hormone Production

Medium containing hydrocortisone at concentrations in the range of 5×10^{-10} to 5×10^{-4} M was added to cells in early stationary phase. Maximum stimulation was observed by 110 hr. The dose-response relationship is shown in Fig. 2. Stimulation of GH production by hydrocortisone was observed at a concentration as low as 5×10^{-8} M. A standard concentration of 3×10^{-6} M was chosen for subsequent experiments.

Duration of Stimulation

The production of GH by hydrocortisone-treated cells was followed over an 11-day period. The results are shown in Table II. In the presence of hydrocortisone, the rate of production of GH

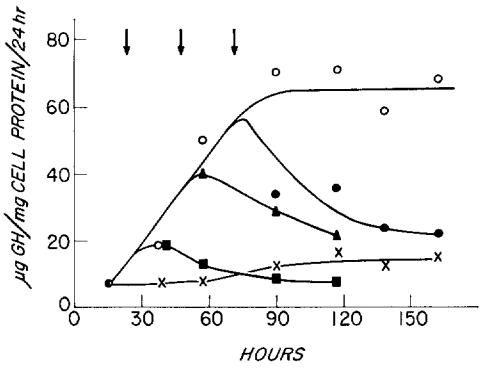

FIGURE 3 Effect on GH production of removal of HC. This experiment was performed using replicate dishes in parallel with the experiment shown in Fig. 1. All dishes, except controls, received medium containing HC (3×10^{-6} M) at zero time and were treated as described in Fig. 1. At either 27, 48, or 72 hr, the dishes were washed three times with saline and medium lacking HC added. The arrows indicate times of removal of HC. (×—× = −HC throughout, ○—○ = +HC throughout; ■—■ = −HC at 27 hr; ▲—▲ = −HC at 48 hr; ●—● = −HC at 72 hr).

was four to eight times higher than that in control cells throughout the course of the experiment. The increase in the rate of GH production by the control cells during this experiment will be considered in the Discussion. In other experiments performed under similar conditions, we have occasionally observed a drop in the rate of GH production in stimulated cells following the attainment of the maximum rate. In no case, however, did the stimulated rate fall to less than three times that in control cells.

TABLE III

Intracellular vs Extracellular Levels of Growth Hormone in Control and Hydrocortisone-Treated Cells

Treatment	Growth hormone in medium	Growth hormone in cells
	μg GH/mg cell protein/24 hr	*μg GH/mg cell protein*
−HC	17	0.19
+HC	98	0.96

Medium either containing HC (3×10^{-6} M) or lacking HC was added to cells in the early stationary phase. Medium was changed every 24 hr. At 96 hr, medium was collected from control and HC-treated cells, and the cells washed three times with saline. The cells were then scraped from the dishes, suspended in 3 ml of isotonic saline, and treated for 5 min at 1–2°C in a Raytheon Model DF101 sonic oscillator. Microscopic examination of the sonicates revealed that less than 1% of the cells had remained intact. After removal of aliquots for determination of protein, the cell sonicates and the 72–96-hr medium were assayed for GH. A control experiment revealed that the observed low intracellular concentrations of GH were not due to interference by intracellular non-GH cell protein with the assay system. When GH was added to sonicated GH_3 cells, the recovery of the added GH, measured immunologically, was 93%.

Intracellular vs Extracellular Levels of Growth Hormone

Intracellular GH caused the same amount of complement to be fixed at antigen-antibody equivalence with antiserum to GH as did either GH secreted into the medium or the rat GH standard. The results presented in Table III show that intracellular levels of GH in unstimulated and stimulated cells are small compared to the amounts appearing extracellularly during a 24-hr interval. It is also seen that intracellular levels reflect the rate of GH production by the cells, corresponding in both stimulated and unstimulated cells to the amount appearing in the medium during an interval of approximately 15 min.

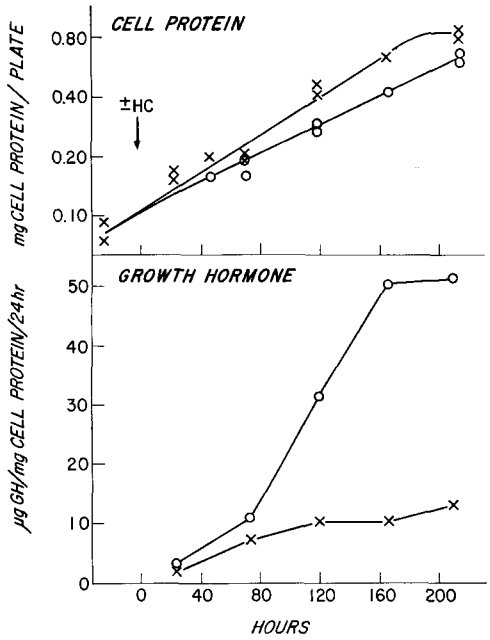

FIGURE 4 Effects of HC on growth and GH production of GH_3 cells. Cells were inoculated in replicate dishes 3 days before the addition of HC (3×10^{-6} M) to some of the dishes. Conditions were those described in Fig. 1, except the medium was collected and changed every 2 days. (×—× = −HC, ○—○ = +HC).

Effect on Growth Hormone Production of Removal of Hydrocortisone

The effect of the removal of hydrocortisone at various times is shown in Fig. 3. Removal at 27, 48, or 72 hr caused the rate of GH production to fall toward the rate in control cells.

Effect of Hydrocortisone on Growth of GH_3 Cells

It was of interest to determine whether hydrocortisone stimulates the production of GH by some specific mechanism, or whether it simply causes a general stimulation of the metabolism of GH_3 cells, thereby leading indirectly to an increase in GH production. This question was investigated by determining the effect of hydrocortisone on the growth of GH_3 cells. The results of such an experiment are shown in Fig. 4.[6] They demonstrate that addition of hydrocortisone to the culture medium of cells growing exponentially with a population-doubling time of about 60 hr caused the cells to enter a new state of exponential growth characterized by a population-doubling time of about 90 hr. Fig. 4 also shows the stimulation by hydrocortisone of the rate of GH production in these cells. It should be noted that the rate of GH production in the unstimulated cells during exponential growth was lower than that observed in unstimulated cells in stationary phase. However, although a somewhat longer time is required to reach a maximum rate, the lag period and the maximum stimulation of GH production are similar to those observed in cells in stationary phase (Fig. 1). Similar effects of hydrocortisone on the growth and GH production of GH_3 cells have been observed in two other experiments.

TABLE IV

Effects of Inhibitors of Protein Synthesis on the Incorporation of Labeled Amino Acids into Protein

Treatment	^{14}C-amino acid incorporation
	cpm/mg protein
None	324×10^2
Cycloheximide	24.0×10^2
Puromycin	5.39×10^2

Cycloheximide (3.6×10^{-5} M) or puromycin (3.7×10^{-4} M) was added to the medium of GH_3 cells. 30 min later, the volume of medium in each dish was reduced to 2 ml, and about 2.6×10^6 cpm of ^{14}C-labeled amino acids were added. After a 70-min incubation, incorporation into TCA-insoluble material was measured as described in Materials and Methods. Replicate dishes were used for the measurement reported in Tables IV and V.

[6] Since the collection intervals employed in this experiment were not negligible compared to the doubling time for cell protein, it was necessary to calculate the specific rates of GH production on the basis of the *average* values for cell protein during each interval. For cells growing exponentially, the average value of the cell protein per dish over an interval of time from 0 to t ($\overline{N}$) can be calculated as follows: Let N = cell protein/dish. At a time t', $N(t') = N(0) \exp(t' \ln 2/t_2)$, where t_2 is the doubling time for N. $\overline{N} = \int_0^t N(t')dt' / \int_0^t dt'$. Evaluating the integrals leads to the relation: $\overline{N}/N(t) = [1 - \exp(\tau)]/\tau$, where $\tau = t \ln 2/t_2$. The calculated values for $\overline{N}/N(t)$ in this experiment are 0.77 and 0.84 for cells growing in the absence and preesenc of hydrocortisone, respectively.

TABLE V

Effects of Inhibitors of Protein Synthesis on Growth Hormone Production by Control and Hydrocortisone-Treated Cells

Treatment	Growth hormone production	
	0–12 hr	12.5–24.5 hr
	μg GH/mg cell protein/24 hr	
−HC	7.4 ± 0.7	—
−HC + cycloheximide	—	< 0.25
−HC + puromycin	—	0.50
+HC	45 ± 5.0	—
+HC + cycloheximide	—	0.60
+HC + puromycin	—	0.56

Cells were inoculated heavily into replicate dishes. 3 days later, medium containing either HC (3×10^{-6} M) or no HC was added to the dishes. Medium was changed each day for 4 days. On the 4th day at zero time (90 hr after HC had first been added), medium which had been equilibrated at 37°C in a 5% CO_2 atmosphere was added to each dish. At 12 hr, the medium was collected and saved for GH assay, and equilibrated medium with the indicated additions was added. 30 min later (12.5 hr), this medium was removed and discarded, and fresh equilibrated medium with the same additions was added. 12 hr later (24.5 hr), this medium was collected and saved for GH assay, and the dishes were washed and frozen for the determination of cell protein. At each medium change throughout the experiment, medium containing HC was added to the appropriate dishes. Concentrations used were: cycloheximide, 3.6×10^{-6} M; puromycin, 3.7×10^{-4} M. The results of the GH assay of the 0–12 hr medium from dishes which received either cycloheximide or puromycin at 12 hr were averaged. The range of the results is shown.

Requirement for Protein Synthesis for Production of Growth Hormone

Cycloheximide and puromycin have been shown to suppress protein synthesis by different mechanisms. Cycloheximide causes a sharp decrease in the rate of peptide-bond formation (13). In the presence of puromycin peptide-bond formation continues, but results in the release of acid-soluble peptidyl-puromycin fragments (14). Table IV shows that, following a 30-min preincubation with inhibitor, cycloheximide (10 μg/ml, 3.6×10^{-5} M) or puromycin (200 μg/ml, 3.7×10^{-4} M) inhibited the incorporation of labeled amino acids into TCA-precipitable material by 93 and 98%, respectively. Table V shows that either cycloheximide or puromycin at these concentrations suppressed both stimulated and unstimulated rates of GH production during a 12-hr period by at least 94%.

DISCUSSION

We have referred to the experimental measurements of the rate of appearance of GH in the medium of GH_3 cells, and the increase in this rate in the presence of hydrocortisone, as "rate of production" and "increased rate of production" of GH, respectively. The justification for the use of this terminology will now be considered.

We propose the following hypothesis: The rate of appearance of GH in the medium of either unstimulated (−HC) or hydrocortisone-treated (+HC) GH_3 cells is an accurate reflection of the rate at which GH is being synthesized by these cells. This hypothesis depends upon at least two other propositions concerning both −HC and +HC GH_3 cells: (*a*) There is no large intracellular pool of GH. There is also no large intracellular pool of GH precursor which must be processed in some fashion before it is secreted into the medium as GH. (*b*) GH is secreted so rapidly after its synthesis that it almost completely escapes intracellular degradation.

The finding that the amount of intracellular GH in either −HC or +HC cells is equal to the amount appearing in the medium in about 15 min (Table III) shows that there is no large intracellular pool either of GH or of GH precursor which is immunologically identical to GH. Furthermore, the finding that the intracellular material caused as much complement to be fixed at antigen-antibody equivalence as did either secreted GH or the rat GH standard implies that there is no large intracellular pool of immunologically nonidentical, but partially cross-reacting, GH precursor. Had a pool of GH precursor of this type existed, it would have inhibited the homologous GH-antiGH reaction, thus leading to a decrease in the amount of complement fixed at equivalence (15).

The average rate of turnover of cell protein in mammalian cell cultures has been found to be about 1% per hr, whether the cells are in the exponential or stationary phase of growth (16). Hence, if it is assumed that intracellular GH turns over no faster than this, the finding concerning the

relative amounts of intracellular and secreted GH referred to above also lends support to the hypothesis that GH escapes intracellular degradation.

Finally, the finding that the suppression of protein synthesis by either of two agents having different modes of action causes a concomitant suppression of the appearance of GH in the medium of either −HC or +HC cells (Table V) lends strong direct support to the original hypothesis that the rate of appearance of GH in the medium of both −HC and +HC cells accurately reflects the rate of GH synthesis in these cells.[7]

We have chosen the neutral term "GH production" over the more specific term "GH synthesis" to report experimental observations as a reflection of the fact that, although the results reported here support the original hypothesis, it has not yet been proven.

Previous studies of the effect of hydrocortisone on GH production and/or secretion have tended to yield opposite results, depending upon whether they were performed in vivo or in vitro. For example, following a 2-day exposure to hydrocortisone, growth hormone levels in human plasma have been found to be *lower* than those found in control subjects (17). Such an effect may be an indirect one, however, since evidence has been presented that a similar effect observed upon administration of hydrocortisone to rats is mediated by a decrease in GH-releasing activity in the hypothalamus (18). Hydrocortisone has also been shown to *decrease* the release of GH from rat pituitary fragments incubated for short periods (60 hr) in vitro (19). It is likely, however, that in this study little or no net synthesis of GH occurred during the experiment. On the other hand, in a study using long-term (1–6 months) primary cultures of normal monkey pituitary glands, Kohler and coworkers (7) have observed a *stimulation* of GH production by hydrocortisone whose kinetics and magnitude are similar to those reported here. Thus, it may be that direct stimulation of GH production by hydrocortisone does occur in vivo, but it is masked by a decreased rate of GH release mediated by the hypothalamus.

[7] An alternative interpretation of this experiment is that GH is synthesized as a totally noncross-reacting precursor, and that the conversion of this precursor to GH requires protein synthesis. However, this interpretation depends upon two *ad hoc* assumptions, and is thus considerably less satisfactory than the one given above.

The large (five- to sevenfold) increase in the rate of GH production in unstimulated cells during the course of the experiments reported in Table II and Fig. 4 has been seen in virtually all the experiments we have performed in which the cells were growing exponentially at the start of the experiment. Similar increases were reported earlier by Tashjian et al. (9). An analogous effect has also been observed in mouse fibroblasts, where a 15-fold increase in the production of collagen is observed when the cells enter stationary phase (20). The fact that the rate of GH production by GH_3 cells increased when the cells were still growing exponentially (Fig. 4) shows both that cell division does not preclude GH synthesis and that increased production of GH can occur during increases in the population density of the cells, as well as during a change in their growth state (Table II).

Addition of hydrocortisone to the medium of exponentially growing GH_3 cells has been observed to decrease the growth rate of the cells. A similar effect of hydrocortisone on the growth rate of HeLa cells in monolayer culture has been observed (21). This finding implies that the stimulation of GH production by hydrocortisone is not merely a reflection of a generalized stimulation of the metabolism of these cells. This concept is supported further by the finding that hydrocortisone causes a decrease in the incorporation by GH_3 cells of labeled amino acids into cellular protein (F. C. Bancroft and A. H. Tashjian, Jr., unpublished observations).

The growth curve shown in Fig. 4, together with the measured amount of GH appearing in the medium per dish, can be used to calculate a value for the fraction GH represents of the total protein synthesized by either unstimulated (−HC) or stimulated (+HC) GH_3 cells. In the interval from 146–188 hr, −HC and +HC cells produced 9 and 33 μg per dish, respectively, while the total cell protein per dish increased by 322 μg (−HC) and 132 μg (+HC). If one assumes a constant average rate of turnover of cell protein of 1% per hour (16) and no intracellular turnover of GH, and uses the relation derived above[6] to calculate the total amount of intracellular protein which was synthesized and degraded in this time interval, the calculated values for the fraction of the total protein synthesized represented by GH are 2% (−HC) and 14% (+HC). By comparison, it has been estimated that collagen represents about 7 and 12% of the protein synthesized by a mouse fibroblast line (22) and a human fibroblast line

(23), respectively, when the cells are in the stationary phase of growth.

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Note Added in Proof

Since submission of this manuscript, we have found that the GH_3 cells also produce the protein hormone prolactin. The production of prolactin by GH_3 cells is not stimulated by hydrocortisone. This finding is a further indication of the specificity of the effect of hydrocortisone on GH production by GH_3 cells.

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