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CONTROL OF THE PRODUCTION OF TWO PROTEIN HORMONES BY RAT PITUITARY CELLS IN CULTURE*

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SUMMARY

A clonal strain of rat pituitary tumor cells (GH_3) has continued to produce the pituitary protein hormones growth hormone (GH) and prolactin during 5 years of continued growth in monolayer culture. Studies of the effects of external stimuli have indicated that, in spite of the physical similarity of these protein hormones (each is a single polypeptide of molecular weight ~23,000), their production is controlled by different mechanisms. Addition of hydrocortisone (HC) (3×10^{-6} M) to the growth medium leads, after a lag of 12 to 24 hr, to an increased relative rate (rate in experimental cells divided by rate in control cells) of GH production. The relative rate reaches a maximum of 5 to 8 at 30 to 100 hr. Stimulation by HC of GH production is observed in cells growing in either the stationary or the exponential phase of growth. Indirect estimates indicate that, in exponentially growing cells, GH represents about 2% and 14% of the total protein synthesized by control and fully stimulated cells, respectively. Maintenance of the stimulated state requires HC. HC decreases both the growth rate of GH_3 cells and their incorporation of amino acids into acid-insoluble material. At the same time that HC stimulates GH production it decreases the relative rate of prolactin production to about 0.2 to 0.3. On the other hand, addition of acid extracts of bovine hypothalamus, cerebral cortex, kidney, or liver (0.3 to 1.0 mg of protein per ml) to the medium leads to an increase of the relative rate of prolactin production to 6 to 9, while decreasing the relative rate of GH production to about 0.5. Chromatographic fractionation of simple extracts of bovine liver has yielded a macromolecular, heat-labile fraction exhibiting these effects at a concentration as low as 20 μg per ml. GH_3 cells which have been adapted to growth in suspension culture produce both GH and prolactin. HC is observed to stimulate GH production and suppress prolactin production by cells growing in this state, without affecting the growth rate of the cells.

The control of the synthesis of particular proteins by animal cells is at present poorly understood. The difficulty in obtaining knowledge in this area arises partially from the structural complexity of eukaryotic cells. However, it also arises from the complexity of the environment in which animal cells are naturally found, i.e. the intact animal. It seems clear that, if control of protein synthesis in animal cells is to be understood, investigations must be carried out using simplified model systems.

Due largely to the efforts of Sato and colleagues (1), it is now possible to grow clonal strains of animal cells which maintain in culture the ability to carry out specialized functions typical of their organ of origin. At present, growth for long periods of time (at least 5 years) of functional cells in culture appears to require that they be neoplastic. But this seems a small price to pay for the ability to study animal cells under simple and well defined conditions, similar to the conditions employed so profitably in studies of the control of protein synthesis in bacteria.

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We have been investigating the control of the production (i.e. synthesis and secretion) of GH* and prolactin by a clonal strain of rat pituitary tumor cells (GH_3). In this report we describe studies of the control of the the production of GH and prolactin by two different sorts of external stimuli—HC and tissue extracts. We have found that while HC stimulates GH production it suppresses prolactin production. Conversely, a macromolecular, heat-labile fraction prepared from various tissues has been found to stimulate prolactin production, while suppressing GH production.

We believe that the ability to stimulate the production of one exportable protein, while suppressing the production of another, makes the GH_3 cells a useful model system in which to study the synthesis and secretion by animal cells of proteins destined for export. Such investigations should complement the elegant studies by Tomkins and co-workers (2) of the control by dexamethasone (a synthetic analogue of HC) of the synthesis by rat hepatoma cells in culture of an intracellular enzyme, tyrosine aminotransferase.

Methods and Materials

Culture of GH_3 cells. The method of culture of the GH_3 cells in monolayer has been described in detail previously (3). Cultures were grown in plastic tissue culture dishes containing 3 ml (unless otherwise specified) of Ham's F10 medium (4) supplemented with 15% horse serum and 2.5% fetal calf serum (complete F10). 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 start of an experiment.

The method of culture of the GH_3 cells in suspension has also been described in detail (5). The cells were grown in spinner bottles at 36.5°C in Eagle's minimal essential medium (Spinner) (6) supplemented with 15% horse serum and 2.5% fetal calf serum.

Measurement of GH and prolactin. GH and prolactin were assayed immunologically in culture medium or in cell homogenates by the method of microcomplement fixation (7). Details have been described previously of the specific assay methods for GH (3, 8) and prolactin (9). The lower limits of detection by this method for both GH and prolactin in tissue culture medium are 0.025 to 0.10 μg per ml. The reproducibility of the assay for either hormone as performed in these experiments is ±15 to 20%.

Measurement of cell protein. Cell protein was measured by the method of Lowry et al. (10).

Incorporation of ^{14}C-labeled amino acids into cell protein. Approximately 2.6×10^6 cpm (2 μCi) of ^{14}C-reconstituted protein hydrolysate were added to the medium (2 ml) of each dish. Incorporation of ^{14}C-amino acids into TCA-insoluble material was determined as described previously (11).

Preparation of bovine tissue extracts. A detailed description of the preparation of crude extracts of bovine hypothalamus, cerebral cortex, liver, and kidney has been given (9). In the present experiments most of the studies were performed with liver extract. Following preparation of an acetone powder of fresh bovine liver, an acidic extract was prepared using 0.1 N HCl (9). This acid extract was adjusted to pH 7.5, the precipitate which formed was removed by centrifugation, and the neutral supernatant solution was designated crude liver extract. It was characterized further by gel filtration on Sephadex G-100, dialysis, and heating, as described in the text. The protein concentration of the extract was determined by the method of Lowry et al. (10).

Materials. Tissue culture dishes (50 × 15 mm) were purchased from Falcon Plastics. Ham's F10 medium and Eagle's minimal essential medium (Spinner) were obtained from Grand Island Biological Company. Hydrocortisone sodium succinate (Solu-Cortef) was obtained from the Upjohn Company. A mixture of ^{14}C-labeled amino acids (^{14}C-reconstituted protein hydrolysate, 103 to 330 mCi per mmole a.a., 100 μCi per ml) was purchased from Schwarz BioResearch, Inc. Cycloheximide and puromycin dihydrochloride were purchased from Nutritional Biochemicals Corporation.

Results

Origin and characteristics of the GH_3 strain of cells. The GH_3 cell strain was originated in 1965. The cells came from rat pituitary tumor MtT/W5, which has been carried in female Wistar/Furth rats since its induction in a female

* The abbreviations used are: GH, growth hormone; TCA, trichloroacetic acid; C′, complement; HC, hydrocortisone sodium succinate.

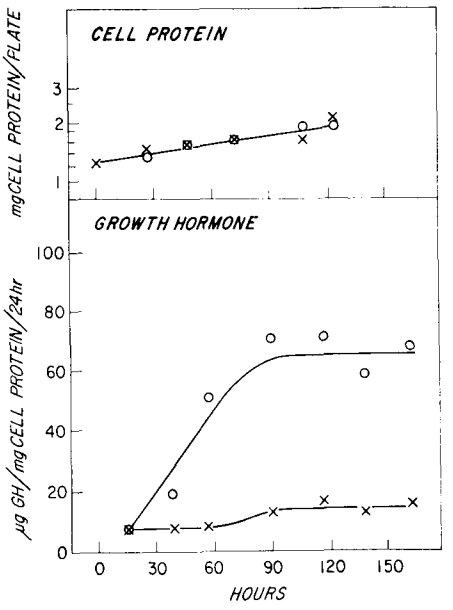

FIG. 1. Stimulation of GH production by HC. Replicate dishes containing cells in early stationary phase were used. At zero time fresh medium containing either HC (3×10^{-6} M) or lacking HC was added to each dish. Every 24 hr the medium was collected from an HC-treated and a control dish and frozen for 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. (Reproduced from Bancroft, F. C., L. Levine, and A. H. Tashjian, Jr. 1969. J. Cell Biol. 43: 432.)

rat of this strain (via X-irradiation of the head) in 1958 (12). The method of alternate culture and animal passage developed by Sato and coworkers (1) was used to establish the primary culture from which the GH_3 strain was isolated (3). Following various procedures designed to eliminate selective overgrowth by connective tissue cells (3), the GH_3 clone was isolated by repeated use of the technique of Puck et al. (13).

The GH_3 cells have a round, epithelioid appearance (3). They have a large distinct nucleus, generally containing a single large nucleolus. The cells grow in fairly well defined colonies, which do not form complete monolayers even after prolonged culture (3). When plated sparsely in complete F10 medium, GH_3 cells, after a lag of 1 to 2 days, grow exponentially with a cell protein-doubling time of about 60 hr. They eventually reach a state where cell protein stops increasing exponentially and either levels off or increases at a greatly reduced rate. In this state of cell growth, which we refer to as early stationary phase, one GH_3 cell contains about 1.7×10^{-7} mg of cell protein.

The GH_3 cells were adapted to growth in suspension culture in April 1969 (5). A stock of the cells has been grown continuously (except for 2 weeks of storage at −196°C) in suspension since then. In this state the cells grow from 1 to 2 $\times 10^5$ cells per ml to 8 to 10 $\times 10^5$ cells per ml with doubling times for cell number and cell protein of about 45 hr and 60 hr, respectively (5).

The modal number of chromosomes in the GH_3 cell strain is 69 (range = 65 to 73) (14), compared to a normal number for the rat of 42.

At the time that the experiments reported here 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.

Production of GH and prolactin.* It has been shown previously that the method of microcomplement fixation can be used to measure both growth hormone (8) and prolactin (9). It has also been shown that rat GH does not fix C′ with anti prolactin, and rat prolactin does not fix C′ with anti-GH (9). Thus each hormone can be measured specifically when both are present in the same sample.

GH_3 cells in monolayer culture have been found to produce both growth hormone and prolactin (9). The immunological evidence that these products are indistinguishable from the authentic hormones has been described previously (3, 9). In addition, rats bearing a tumor derived from GH_3 cells grow to enormous size and have stimulated mammary glands, indicating that the hormones produced by the cells are biologically active as well (3, 9).

When the GH_3 cells are in the early stationary phase of growth they produce 10 to 30 μg of GH per mg of cell protein per 24 hr (11). GH production is generally lower in cells which are growing exponentially than in cells in early stationary phase (11). Prolactin production is

* The production of either GH or prolactin is defined as the amount of the hormone which accumulates in the growth medium during a given period of time, divided by cell protein. Hence each reported value for production represents the average specific rate of appearance of the hormone in the medium.

usually about the same as GH production, but for reasons not clearly understood it is sometimes observed to be considerably lower or higher than GH production.

When the GH_3 cells are grown in suspension culture, they continue to produce both GH and prolactin (5).

Effects of HC on GH and prolactin production¶. The effect of the addition of HC (3×10^{-6} M) to the medium of GH_3 cells in the early stationary phase of growth is shown in Figure 1. It is seen that following a lag period of about 24 hr GH production in HC-treated cultures increased compared to that in control cultures. At about 90 hr, GH production in the treated cultures reached a maximum rate about 5 times that in the controls.

Protein synthesis is required for the continued production of GH in either unstimulated or fully stimulated cells. It was found that cycloheximide (3.6×10^{-5} M) or puromycin (3.7×10^{-4} M), after a 30-min preincubation, inhibited the incorporation by GH_3 cells of labeled amino acids into TCA-insoluble material by 93% and 98%, respectively. Either cycloheximide or puromycin at these concentrations suppressed both unstimulated and stimulated rates of GH production by a least 95% (Table 1).

Intracellular levels of GH in control and HC-treated cells are equal to the amount of GH produced by the cells in about 15 min (Table 2). Similar measurements of prolactin have indicated that the intracellular levels of prolactin in control cells are equal to the amounts of prolactin produced in about 1 to 2 hr (9). Thus the GH_3 cells do not appear to store large quantities of either protein hormone.

The specificity of the stimulation by HC of GH production by GH_3 cells was examined by studying the effects of HC on protein synthesis, growth rate, and prolactin production.

HC (3×10^{-6} M) caused a progressive decrease in the incorporation by GH_3 cells of labeled amino acids into TCA-insoluble material, while it stimulated the rate of GH production (Table 3). Assuming that this effect is not due to a decrease by HC of the permeability of the cells to amino acids, this result implies that HC simultaneously stimulates GH production and suppresses general protein synthesis by the GH_3 cells.

TABLE 1

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. Three 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. Thirty minutes later (12.5 hr) this medium was removed and discarded, and fresh equilibrated medium with the same additions was added. Twelve hours 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^{-5} M; puromycin, 3.7×10^{-4} M. The results of the GH assay of the 0 to 12 hr medium from dishes which received either cycloheximide or puromycin at 12 hr were averaged. The range of the results is shown. (Reproduced from Bancroft, F. C., L. Levine, and A. H. Tashjian, Jr. 1969. J. Cell Biol. 43: 432.)

The effect of HC (3×10^{-6} M) on the growth of GH_3 cells in the exponential phase is shown in Figure 2. It is seen that HC increased the doubling time for cell protein by about 50%, while it stimulated the rate of GH production.

The specificity of the stimulation of GH production is demonstrated most convincingly by the differential effects of HC on the production of GH and prolactin. HC (3×10^{-6} M) was observed to cause a progressive suppression of prolactin production while it caused a progressive stimulation of GH production (Fig. 3). By 110

¶ The experiments reported in this section were performed using GH_3 cells grown in monolayer in complete F10 medium, except where otherwise specified.

TABLE 2

INTRACELLULAR VERSUS 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 were 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 to 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%. (Reproduced from Bancroft, F. C., L. Levine, and A. H. Tashjian, Jr. 1969. J. Cell Biol. 43: 432.)

hr, prolactin and GH production in the HC-treated cultures were about 25% and 800%, respectively, of the production in control cultures.

Effects of HC on GH and prolactin production similar to those described above for GH_3 in monolayer were observed when the cells were grown in suspension culture (Fig. 4). A lag of 24 to 48 hr was observed. Maximum stimulation of GH production was reached at about 60 hr, when GH and prolactin production by the HC-treated cells were about 700% and 20%, respectively, of the production by control cells. It is interesting to note that HC had no effect on the growth rate of GH_3 cells growing in suspension (Fig. 4).

The stimulation by HC of GH production has a rather long lag period. By comparison, Tomkins and co-workers have found that dexamethasone stimulates levels of tyrosine aminotransferase in cultured rat hepatoma cells with a lag of about 2 hr, while maximum stimulation is reached in 5 to 8 hr (2).

In order to employ inhibitors of protein and RNA synthesis in further studies of the events occurring during the lag period in GH_3 cells, it is desirable to search for conditions which shorten the time scale of the HC effect. We have found that the lag period can be shortened by simply changing the medium more frequently. Such an experiment, using cells in the exponential phase, is illustrated in Figure 5. It is seen that when a collection interval of 6 hr was employed a significant stimulation of GH production was observed by 12 hr. Furthermore, a maximum stimulation of about 4-fold was observed by about 30 hr. It should also be noted that the rate of production of GH by the control cells increased about 3-fold during this experiment. It is not clear why frequent medium changes should have these effects. Studies of these phenomena, and attempts to shorten the lag period still further, are currently in progress.

Effects of tissue extracts on GH and prolactin production. We have previously reported that crude extracts of bovine hypothalamus, cerebral cortex, liver, and kidney contain material that stimulates the production of prolactin and sup-

TABLE 3

EFFECTS OF HC ON PROTEIN SYNTHESIS AND GH PRODUCTION BY GH_3 CELLS*

Time	Relative† GH Production (+HC:−HC)	Relative‡ a.a. incorporation (+HC:−HC)
hr		
7.5	—	0.93
33	1.8	0.72
51	1.4	0.68
73	1.7	0.68
98	2.7	0.56

* Cells were inoculated sparsely in replicate dishes 3 days before the addition at zero time of HC (3×10^{-6} M) to some of the dishes. Conditions for measurements of GH production were as in Figure 1. At the indicated times incorporation of ^{14}C-labeled amino acids into TCA-insoluble material was measured as described in "Methods and Materials." Cell protein increased exponentially in HC-treated and control dishes during the course of the experiment.

† Production in HC-treated cultures divided by production in control cultures. Absolute values of GH production were in the range of 2 to 28 μg of GH per mg of cell protein per 24 hr.

‡ Incorporation of ^{14}C-amino acids into TCA-insoluble material in HC-treated cultures, divided by incorporation in control cultures. Absolute values of incorporation were in the range 4×10^3 to 27×10^3 cpm per mg of cell protein.

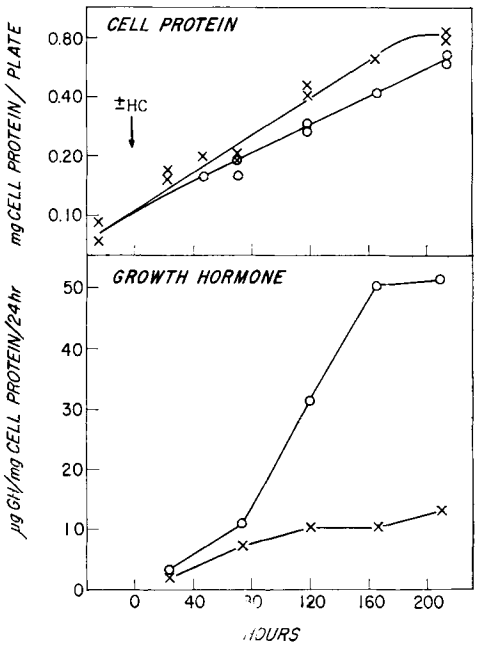

FIG. 2. Effects of HC on growth and GH production of GH_3 cells. Cells were inoculated in replicate dishes 3 days before the additions of HC (3×10^{-6} M) to some of the dishes. Conditions were those described in Figure 1, except that the medium was collected and changed every 2 days. ×——×, −HC; ○——○, +HC. (Reproduced from Bancroft, F. C., L. Levine, and A. H. Tashjian, Jr. 1969. J. Cell Biol. 43: 432.)

presses GH production (9). The differential effects of hypothalamic extract on prolactin and GH production are shown in Figure 6. The effects of crude liver extract on hormone production by GH_3 cells were qualitatively similar to those of hypothalamic material (9). Because of its greater availability, we have begun to characterize the active principle in the liver extract. At dose levels of 100 to 400 μg of protein per ml medium, crude liver extract consistently stimulates prolactin production by 3- to 5-fold during the first 3 days after its addition and 5- to 10-fold during days 3 to 7 after its addition. During these two periods GH production is consistently reduced by about 50% and 80%, respectively. The effect of crude liver extract on cell growth is somewhat variable and does not correlate with effects on hormone production; in some experiments there was no effect on cell growth as measured by increase in protein, in some experiments a 30 to 50% decrease, and in some a 20 to 50% increase.

When the crude liver extract was chromatographed on Sephadex G-100, nearly all of the prolactin-stimulating material was recovered in a macromolecular fraction appearing near the void volume of the column (Fig. 7). Table 4 shows the effects of a pool of this early Sephadex fraction on prolactin and GH production. As was observed with the crude extract, production of prolactin was greatly stimulated, while GH production was inhibited. These effects were also observed at lower dose levels with the Sephadex fraction than with the crude extract.

The behavior of the prolactin-stimulating factor during dialysis was consistent with the conclusion drawn from the gel filtration studies, i.e. that the material is a macromolecule. A 2-ml sample of the active Sephadex fraction was dialyzed against 2000 ml of 0.01 M Tris, pH 7.5, for 24 hr at 4°C. All of the prolactin-stimulating activity was recovered from inside the bag.

Figure 8 shows that the prolactin-stimulating activity of liver extract is rapidly lost when the extract is incubated at temperatures of 60°C or greater.

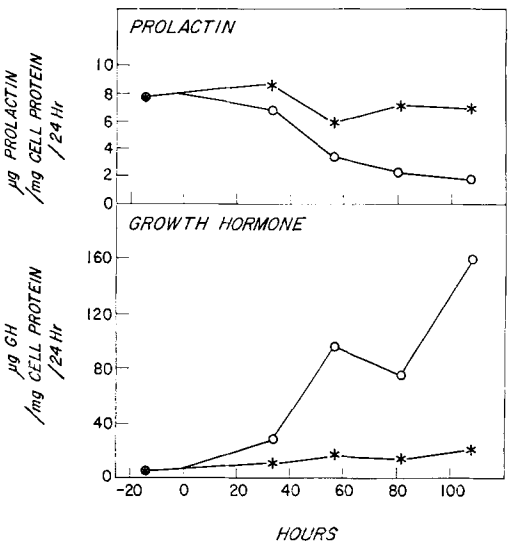

FIG. 3. Effects of HC on prolactin and GH. Duplicate dishes were used for each point. At zero time fresh medium containing either HC (3×10^{-6} M) or lacking HC was added to each dish. Medium was collected at intervals from HC-treated (○) and control (*) dishes and was frozen for hormone assays. These dishes were washed and frozen for determination of cell protein. (Reproduced from Tashjian, A. H., Jr., F. C. Bancroft, and L. Levine, 1970. J. Cell Biol., in press.)

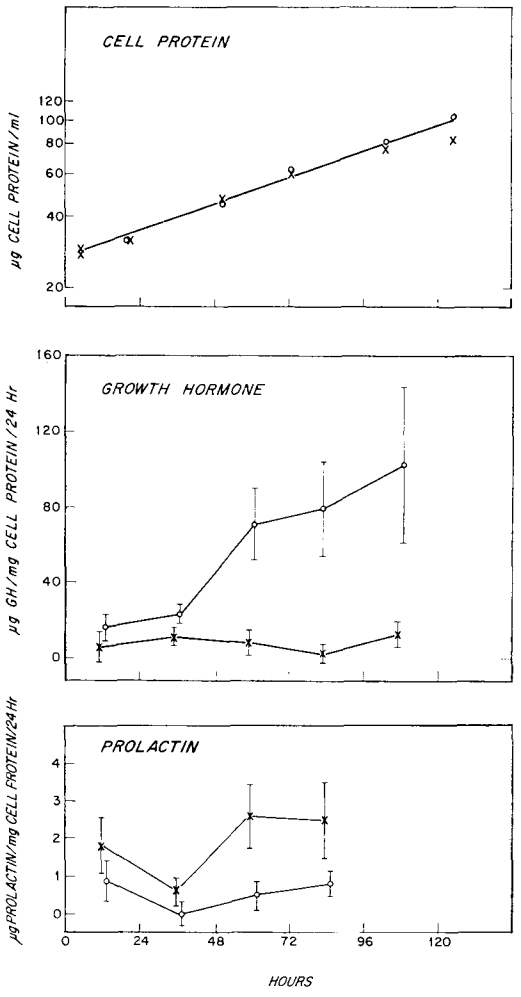

FIG. 4. Effects of HC on growth and GH and prolactin production of GH_3 cells in suspension. At zero time a suspension culture was divided equally between two spinner bottles. One bottle (+HC) received HC at a final concentration of 3×10^{-6} M, and the other (−HC) received an equal volume of saline. Additions were repeated on the 2nd and 4th day of the experiment. Aliquots were removed every 24 hr for assay of cell number, cell protein, and GH and prolactin concentration. GH and prolactin production were calculated from the difference between the concentrations of the hormones measured at the beginning and end of a 24-hr interval, divided by the average cell protein concentration during the interval. The *vertical bars* on each GH and prolactin point represent the uncertainty in the difference between two values each having an uncertainty of ±15%. ×——×, −HC; ○——○ = +HC.

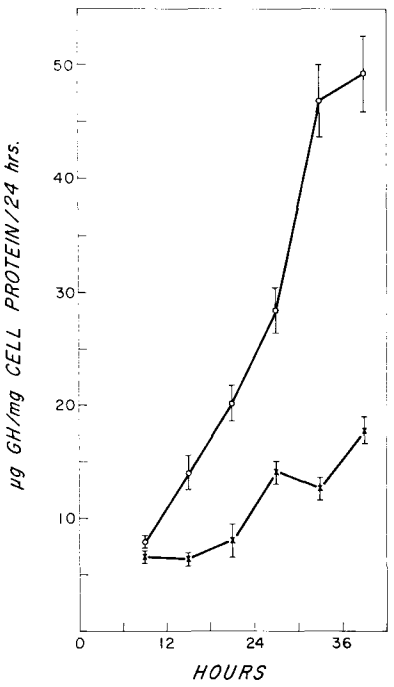

FIG. 5. Effects of frequent medium changes on the stimulation by HC of GH production. Cells, 3×10^5, were inoculated into each of a number of dishes 5 days before the beginning of the experiment. At zero time 2 ml of fresh medium either containing HC (4.5×10^{-6} M) or lacking HC were added to each dish. Every 6 hr the medium was collected from three HC-treated and three control dishes and frozen for GH assay. At the same time, an HC-treated and a control dish were washed twice with isotonic saline and frozen for determination of cell protein. The medium on all remaining dishes was then changed, and medium containing HC was added to the appropriate dishes. Cell protein in HC-treated and control dishes increased exponentially during the course of the experiment. Each *point* represents the mean of GH assays of media collected from the three dishes; the *vertical bars* represent the standard deviation of the mean. ×——×, −HC; ○——○ = +HC.

DISCUSSION

In addition to being pituitary hormones, GH and prolactin are also both authentic proteins. As protein molecules, they bear certain similarities to each other. Thus, rat GH and rat prolactin both have molecular weights of about 23,000 (15, 16). The sequences of human GH (17) and sheep prolactin (18) have been reported. They are both about the same size as the rat hormones, and are

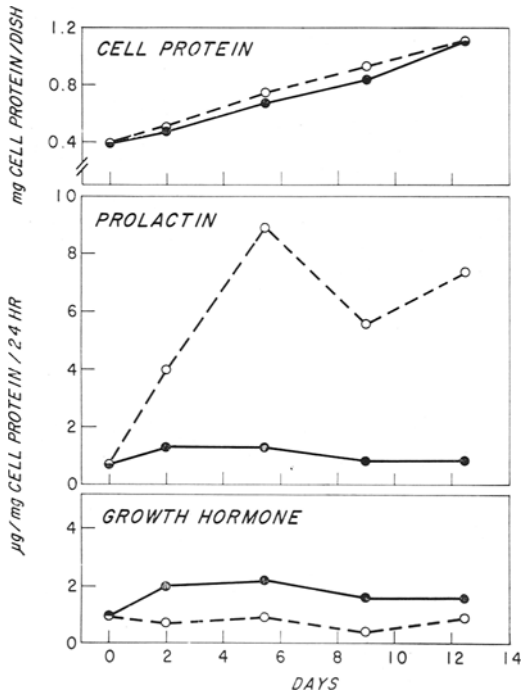

FIG. 6. Effects of bovine hypothalamic extract on cell protein, prolactin, and GH in cultures of GH_3 cells. Duplicate dishes were used for each point. At zero time medium containing either hypothalamic extract (0.14 ml of extract per dish, 1.5 mg of extract protein per dish) or lacking extract was added to each dish. Medium was collected at intervals from experimental (○) and control (●) dishes and was frozen for hormone assays. These dishes were washed and frozen for determination of cell protein. Medium containing hypothalamic extract did not fix C′ with antiprolactin, nor did it interfere with the assay of prolactin in medium. (Reproduced from Tashjian, A. H., Jr., F. C. Bancroft, and L. Levine. 1970. J. Cell Biol., 47: 61.)

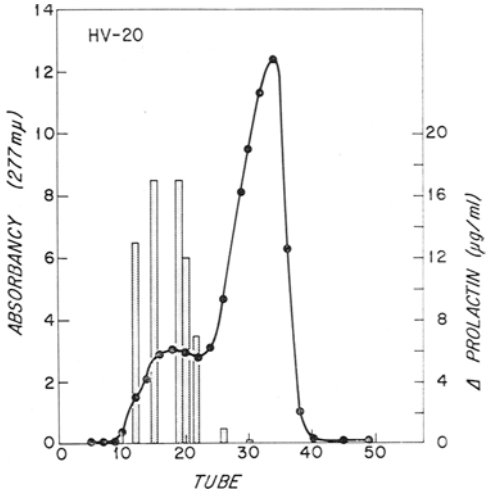

FIG. 7. Elution pattern from Sephadex G-100 of crude extract of bovine liver (equivalent to 40 ml of original extract). The column was 1.9 × 50 cm. Fractions of 3.0 ml per tube were collected at 4°C at a flow rate of 8 to 10 ml per hr. Aliquots from individual tubes were tested on cultures of GH_3 cells for prolactin-stimulating activity. Each aliquot was tested at a dose level of 25 μg of protein per ml of culture medium for a period of 72 hr. The results are given by the *vertical bars* which show the increase in prolactin (*Δprolactin*) in medium above control levels (7 μg per ml). In each case where prolactin production was stimulated, GH production was inhibited by 30 to 50%.

both composed of single polypeptide chains. It seems likely, therefore, that each rat hormone also contains a single polypeptide chain. It is interesting to note that human GH and sheep prolactin also bear close similarities in three regions, corresponding to about 45% of either polypeptide chain (19).

As described above, the GH_3 clonal strain of rat cells has been shown to produce both GH and prolactin. Since the GH_3 strain was derived originally from a single cell, this result suggests that both protein hormones can be produced by the same type of pituitary cell. This conclusion

TABLE 4

EFFECTS OF A MACROMOLECULAR SEPHADEX FRACTION OF BOVINE LIVER ON THE PRODUCTION OF PROLACTIN AND GH BY GH_3 CELLS*

Treatment	Dose	Cell Protein	Prolactin	GH
	μg protein/ml	*mg/dish*	*μg/mg cell protein/24 hr*	
None	—	0.46	10	2.2
Crude extract	400	0.36	92	0.46
Sephadex fraction	275	0.42	190	0.40
	100	0.41	140	0.60
	20	0.44	53	0.76

* Cells in replicate dishes were incubated without extracts or with extracts at the dose levels indicated for a total period of 7 days. The data given in the table are for the interval 96 to 168 hr. At the end of the experiment medium was collected and frozen for hormone assays. The dishes were washed and the cells were frozen for protein determination. The control group is the mean of duplicate determinations; the values for treated groups are from single dishes.

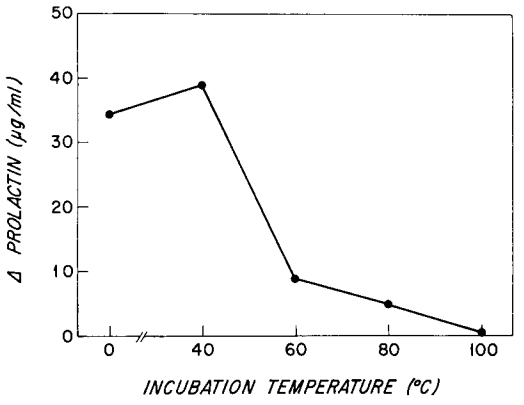

FIG. 8. Effect of heat on the prolactin-stimulating factor in bovine liver. Crude liver extract was neutralized and diluted with an equal volume of 0.1 M Tris, pH 7.5. Equal aliquots were placed at 0°C and in water baths at temperatures of 40, 60, 80, and 100°C for 10 min. Any precipitates that formed on heating were removed by centrifugation and the supernatant solutions were tested for stimulation of prolactin production of GH_3 cells for a 4-day period. Each fraction was tested at a dose level of 100 μg of protein per ml of medium. Each *point* gives the increase in prolactin (*Δprolactin*) in medium above the control level (11 μg per ml).

is strengthened by recent experiments in which GH_3 cells were cloned twice in rapid succession. The resulting clonal line of cells was found to produce both GH and prolactin (20). However, until production of the hormones by individual cells can be measured, it will not be possible to demonstrate conclusively that a single GH_3 cell can simultaneously produce both GH and prolactin. Furthermore, the fact that a single type of neoplastic cell growing in tissue culture produces both GH and prolactin does not necessarily imply that both hormones can be or are produced by the same cell in the pituitary gland in vivo. In any case, it is remarkable that the GH_3 cell strain has retained the ability to produce both hormones during 5 years (~ 720 generations) of growth in culture, in the absence of any known selection pressure in favor of the production of either hormone.

Because experiments can be performed with the GH_3 cells under highly controlled conditions, this cell strain appears to represent an excellent model system to use in the study of the mechanisms which control the production by mammalian cells of secretory proteins. Furthermore, the fact that the cells produce two similar proteins means that the specificity of the effects of external stimuli on these mechanisms can be determined. Thus if a stimulus were observed to increase the production of one protein, while either decreasing or having no effect on the production of the other, this would demonstrate in a quite convincing fashion the specificity of the observed effects.

We have described in this report experiments designed to elucidate some of the factors involved in the effects of two such stimuli, hydrocortisone and tissue extracts. In addition, the partial isolation and characterization of the factor in tissue extracts responsible for these effects have been reported. It remains for the future to elucidate in detail the molecular mechanisms whereby GH and prolactin production by the GH_3 cells are specifically stimulated by hydrocortisone and a macromolecular, heat-labile fraction of tissue extracts, respectively.

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