Cortisol Induction of Growth Hormone Synthesis in a Clonal Line of Rat Pituitary Tumor Cells in Culture

Abstract. *Growth hormone synthesis increased markedly after addition of glucocorticoids at physiological concentrations to cultures of the GH_1 line of rat pituitary tumor cells. Stimulation of hormone protein synthesis by corticosteroids was selective, since (i) the rate of hormone synthesis increased while total protein synthesis decreased, and (ii) cortisol analogs, biologically inactive metabolites, and sex steroids did not induce growth hormone synthesis.*

We have reported stimulation of growth hormone production by cortisol in explant cultures of monkey adenohypophysis and human pituitary adenomas (*1*). To study this effect of cortisol in a more homogeneous cell population, we have utilized the GH_1 clonal line of rat pituitary tumor cells isolated by Yasumura and colleagues (*2*). These cells retain the organ-specific capacity to produce growth hormone during serial propagation. In all experiments replicate cultures of GH_1 cells were grown in 30-ml plastic flasks in culture medium F10 (*3*) supplemented with 15 percent horse serum and 2.5 percent fetal bovine serum. Complete changes of medium were made every 24 hours. Growth hormone was extracted from the cells with 0.02*M* NH_4OH. The growth hormone content of the medium and neutralized cell extracts was measured by a previously described radioimmunoassay (*4*).

When growth hormone levels in the medium were measured, control cultures showed a slow rise in hormone content (Fig. 1). The addition of cortisol (5×10^{-8} to $5 \times 10^{-6}M$) caused marked increases in growth hormone levels after 48 hours of treatment. However, an increased rate of growth hormone synthesis was detected by 24 hours, when the cell content of the hormone was measured. Six replicate cultures of GH_1 cells treated with $5 \times 10^{-7}M$ cortisol for 1 day contained 642 ± 32 (mean $\pm$ S.E) ng of growth hormone per culture, whereas six untreated controls contained 433 ± 29 ng ($P < .01$).

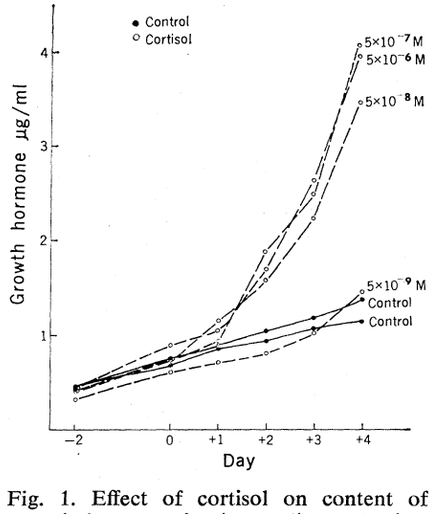

Fig. 1. Effect of cortisol on content of growth hormone in the medium. Cortisol in concentrations indicated was added to replicate cultures of GH_1 cells on day 0, and the medium was changed every 24 hours.

The higher growth hormone values in cortisol-treated cultures were not the result of an increased number of cells, since cortisol at $5 \times 10^{-7}M$ actually slowed the rate of cell replication. After 4 days of cortisol treatment, the number of cells in treated cultures was $3.04 \pm 0.14 \times 10^6$ (mean $\pm$ S.E. for triplicate cultures) compared to $6.81 \pm 0.53 \times 10^6$ for control cultures. Therefore, although growth hormone production per culture was increased three to four fold by cortisol, the actual amount of growth hormone synthesized per cell was increased six to eight times by cortisol treatment.

Once synthesized, growth hormone was rapidly secreted into the medium by GH_1 cells. A comparison of growth hormone levels in cells and medium in control cultures and replicate cultures treated with cortisol for 4 days is shown in Table 1. The observation that storage of growth hormone in the cells was relatively low in comparison to the amount of hormone released into the medium, as well as the magnitude of the growth hormone increase after cortisol, suggested that the effect of cortisol was on growth hormone synthesis rather than release. In support of this hypothesis was the finding that cycloheximide, an inhibitor of protein synthesis, at 0.1 μg/ml in the medium, reversibly blocked the induction of growth hormone by cortisol.

To determine if cortisol stimulated overall protein synthesis in the GH_1 cells, we examined the incorporation of ^{14}C-labeled amino acids (*5*) into protein. Medium containing ^{14}C-labeled amino acid mixture (0.2 μc/ml) was added to treated and control cultures for 1 hour at intervals after the addition of cortisol. Radioactive amino acid incorporation into protein in cell homogenates was measured by the filter paper disk method of Mans and Novelli (*6*), and protein, by the method of Lowry *et al.* (*7*). A significant relative decrease ($P < .05$) in the rate of total protein synthesis was present by 24 hours in the cortisol-treated cultures compared to control cultures (Table 2). Therefore, the induction of growth hormone by cortisol was selective to the extent that growth hormone synthesis increased under conditions where the rate of total protein synthesis was lower than that of control cultures.

The specificity of cortisol as the inducer was examined by testing other groups of steroids at equimolar ($5 \times 10^{-6}M$ to $5 \times 10^{-8}M$) concentrations. Relative potency of several other active steroids compared to cortisol were: dexamethasone, 100 percent; corticosterone, 60 percent; aldosterone, 52 percent; and deoxycorticosterone, 38 percent. The analogs cortisone and 11α-hydroxycortisol were inactive, as were the metabolites of cortisol, 5α- and 5β-dihydrocortisol, and tetrahydrocortisol. Sex steroids such as testosterone, 5α-dihydrotestosterone, progesterone, and estradiol were either inactive or decreased growth hormone production.

Table 1. Comparison of amount of growth hormone in cells and amount secreted into medium by replicate cultures after 4 days of cortisol treatment. The numbers indicate the mean of duplicate determinations.

Cortisol concentration	Growth hormone	
	In cells (ng/culture)	In 24-hour medium (ng/culture)
0	100	2240
0	144	2750
$5 \times 10^{-9}M$	108	2910
$5 \times 10^{-8}M$	598	6880
$5 \times 10^{-7}M$	840	8080
$5 \times 10^{-6}M$	570	7870

Table 2. Incorporation of ^{14}C-labeled amino acids into cell protein in control and cortisol-treated ($5 \times 10^{-7}M$) cultures. Values are given as mean $\pm$ standard error of determinations on groups of three replicate cultures. CPM, counts per minute.

Time (hours)	Control (CPM/mg protein)	Cortisol treated (CPM/mg protein)
0	3680 $\pm$ 66	3480 $\pm$ 263
10	4170 $\pm$ 194	4120 $\pm$ 207
24	5270 $\pm$ 168	3330 $\pm$ 223*
48	4970 $\pm$ 158	3150 $\pm$ 70†

* Significantly different from control group, as determined by Student's *t*-test, with a probability $P < .05$. † $P < .01$.

Therefore, it appeared that only those steroids with biological glucocorticoid and/or mineralocorticoid activity were capable of stimulating growth hormone synthesis.

The results of these and previous studies (*1*) suggest that glucocorticoids at physiological levels have a direct effect on pituitary cells in culture, causing an increase in growth hormone synthesis. These findings are in contrast to the suppressive effect of pharmacological doses of cortisol on growth hormone release (*8*). The GH_1 cells provide a model system with which to study the mechanism of the cortisol-induced synthesis of a specific protein and the control of growth hormone production in vitro.

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References and Notes

1. P. O. Kohler, W. E. Bridson, P. L. Rayford, *Biochem. Biophys. Res. Commun.* **33**, 834 (1968); *Clin. Res.* **16**, 523 (1968); P. O. Kohler, L. A. Frohman, W. E. Bridson, T. Vanha-Perttula, J. M. Hammond, *ibid.* **17**, 288 (1969); W. E. Bridson and P. O. Kohler, in preparation. This material was presented in part at meetings of the American Federation for Clinical Research at Boston, Massachusetts, on 7 December 1968 and at Atlantic City, New Jersey, on 4 May 1969.
2. Y. Yasumura, A. H. Tashjian, G. H. Sato, *Science* **154**, 1186 (1966); A. H. Tashjian, Y. Yasumura, L. Levine, G. H. Sato, M. L. Parker, *Endocrinology* **82**, 342 (1968). GH_1 cells were obtained in 37th passage from the American Type Culture Collection, Rockville, Maryland. After more than 50 passages in our laboratory, GH_1 cells have shown no diminution in the basal rate of growth hormone synthesis.
3. R. G. Ham, *Exp. Cell Res.* **29**, 515 (1963).
4. L. A. Frohman and L. L. Bernardis, *Endocrinology* **82**, 1125 (1968).
5. Reconstructed ^{14}C protein hydrolysate, Schwarz BioResearch, Inc.
6. R. J. Mans and G. D. Novelli, *Arch. Biochem. Biophys.* **94**, 48 (1961).
7. O. H. Lowry, N. F. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
8. A. G. Frantz and M. T. Rabkin, *New Eng. J. Med.* **271**, 1375 (1964); C. A. Birge, G. T. Peake, I. K. Mariz, W. H. Daughaday, *Proc. Soc. Exp. Biol. Med.* **126**, 342 (1967).
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Bivalve Mollusk Burrowing Aided by Discordant Shell Ornamentation

Abstract. *Oblique and chevron-like ridges on the shell surfaces of certain burrowing bivalve mollusks grip the sediment during shell-rocking movements to aid in sediment penetration. These ridges (characterized by steep dorsal slopes and gentle ventral slopes) have evolved through convergence in several families in association with particular behavioral and ecological traits.*

Analysis of mechanical functions of present-day skeletal features can provide important information for interpreting the evolution and paleoecology of fossil groups. Bivalve mollusks, because of their high ecologic and taxonomic diversity in both modern and ancient seas, offer great potential for the functional morphologic approach. One of the most significant shell features of burrowing bivalves is the configuration of surface ornamentation. Oblique and chevron-shaped ridges, especially, have aroused curiosity because they have evolved independently in fossil and living species of several families and because they represent departures from normal shell geometry (*1*). I have analyzed the function of such ridges for three living genera and found that they serve as mechanical aids to burrowing.

Most ridged ornamentation in bivalves follows simple concentric or radial patterns. Concentric ridges are secreted periodically during shell growth by the entire mantle edge (which underlies the shell margin). Radial ridges are the product of continuous secretion by discrete zones of the mantle margin. Unusual ridge patterns of the type considered here (discordant ridges) transect both concentric and radial structures and are secreted by migration of ridge-secreting zones along the mantle edge during growth. Secretion of chevron-shaped discordant ridges (Fig. 1) further requires periodic formation of new ridge-secreting zones at central positions, from which the zones divide and migrate in opposite directions.

In the four living species I have studied (*Tellina similis, Strigilla carnaria*, and *Strigilla mirabilis* of the Tellinacea and *Divaricella quadrisulcata* of the Lucinacea) the ridges are asymmetrical, dorsal slopes being steeper than ventral slopes. Shells of the *Strigilla* and *Divaricella* species are nearly circular in outline, and the ridges form a chevron-like pattern. These species are restricted to clean sand substrata, where wave or current scour commonly necessitates downward movement for maintenance of normal burial depths. They are deep burrowers, living at sediment depths several times their shell lengths. Burrowing paths were studied by time-

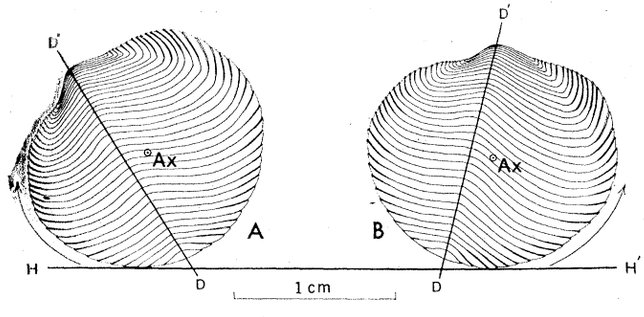

Fig. 1. Mechanism by which chevron-like ridges aid burrowing of *Divaricella quadrisulcata*. (A) Shell orientation at maximum forward rotation. (B) Shell orientation from which forward rotation begins. *Ax*, axis of rotation; *D-D'*, demarcation line between anterior and posterior portions of ridges; *H-H'*, horizontal line. Arrows indicate angle and direction of rotation from each position.

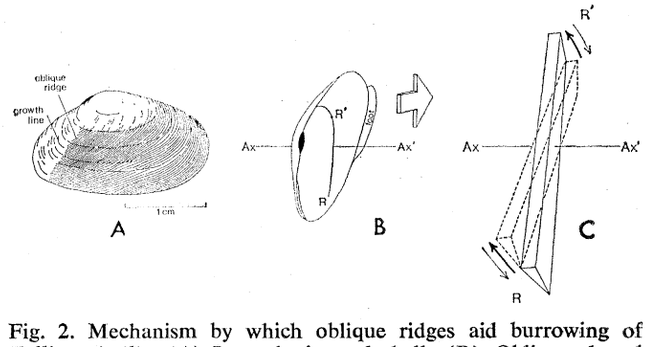

Fig. 2. Mechanism by which oblique ridges aid burrowing of *Tellina similis*. (A) Lateral view of shell. (B) Oblique dorsal view of burrowing animal (schematic), large arrow showing direction of sediment penetration. (C) Diagram of a single ridge of ornamentation, heavy and light arrows denoting rotation opposed by large and small frictional forces, respectively. *Ax-Ax'*, axis of rotation; *R-R'*, single ridge.