DIFFERENT EFFECTS OF HYDROCORTISONE ON THE GROWTH OF HELO CELLS IN MEDIA OF VARIOUS AMINO ACID COMPOSITIONS

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Addition of hydrocortisone to medium with a well-balanced amino acid composition, such as Eagle’s Minimum Essential Medium, inhibited the growth of HeLa cells. However, hydrocortisone had various effects when added to cells cultured in media in which only one amino acid was reduced. Its effect depended on the deficient amino acid: in media of low isoleucine, methionine or phenylalanine content it promoted cell growth, while in media of low lysine or histidine content it had no effect.

In medium containing a low level of methionine (Low Met Med), the free methionine content in the cells increased on addition of hydrocortisone. The incorporation of \(^{35}\)S-methionine into the acid-soluble fraction of these cells also increased on addition of hydrocortisone.

Thus the growth promoting effect of hydrocortisone on cells cultured in Low Met Med may be dependent on increased absorption of limiting amino acid into cells.

A number of hormones have effects on amino acid and protein metabolisms that may be related to the transport, synthesis, and breakdown of these compounds. Studies on the effects of addition of hormones to the medium of cultured cells have provided information on the effects of hormones on individual organs and tissues (1–5). Thus it seemed interesting to investigate the effects of hormones on cells cultured in media containing various amino acid patterns and to compare the results with those on whole animals.

HARPER and his colleagues (6) and NODA et al. (7) reported that glucocorticoid promoted growth of rats on an amino acid imbalanced diet. But the possible secondary effects of hormone (8–9) or hormonal interaction (10) always complicate the interpretation of results in the case of whole animals.

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We are interested in the effect of glucocorticoids on growth promotion of rats on an amino acid imbalanced diet. Cells in tissue culture seem suitable systems to use in investigation of this problem, since they are not under nervous control and other hormones. The effect of a single hormone on them can be tested. The present paper describes the effect of hydrocortisone on cell growth in media of various amino acid balances.

MATERIALS AND METHODS

The cells (strain HeLa), culture procedures and media used were described elsewhere (11). Minimum Essential Medium Eagle with Earle's Balanced Salt Solution (MEM),*1 50% growth medium*2 and media in which only one amino acid was reduced to cause 50% growth reduction were prepared in our laboratory. Hydrocortisone (Sigma, St. Louis, U.S.A.) was added to culture media at final concentrations of 0.02 to 2.00 μg/ml.

For the analysis of free amino acids in the cells, the cells grown on a glass surface were washed twice with 10 ml Ca- and Mg-free salt solution which contains, in percent: NaCl, 1.00; KCl, 0.025; KH₂PO₄, 0.025; K₂HPO₄, 0.144 in redistilled water, and then detached from the glass with 10 ml salt solution containing 0.02% EDTA. Suspended cells were homogenized in a glass homogenizer with a Teflon pestle. Analytical procedure followed the method of Stein-Moore (12), using a Technicon amino acid autoanalyzer.

Incorporation of ³⁵S-methionine (³⁵S-Met) into the cells was measured as follows: The cells were cultured for 4 days in 200 ml flasks containing 1 ml of dialyzed calf serum and 9 ml of low methionine medium (Low Met Med), in which all amino acids except methionine (Met) were at the levels in MEM. The content of Met in Low Met Med was 0.002 mM, a concentration resulting in 50% growth rate of cells in MEM (11). After 4 days' preincubation, the medium was replaced by 9 ml of the fresh Low Met Med containing 0.1 or 1.0 μCi of DL-methionine-³⁵S (Daiichi Kagaku Yakuhin Co., Tokyo; specific activity, 6.2 mCi/mM) with or without 20 μg of hydrocortisone. Dialyzed calf serum (1 ml) was added and incubation was continued for 1, 2 and 3 hr. At each of these intervals, the medium was discarded and the cells were detached from the glass by a Ca- and Mg-free salt solution containing 0.02% EDTA. The detached cells were homogenized with Teflon homogenizer at 0°C. Then the homogenate was rapidly mixed with 1 ml of 50% TCA solution and the mixture was centrifuged at 1,000 × g.

*1 The amino acid composition of Eagle's Minimum Essential Medium is as follows (in mM): Arginine, 0.6; cystine, 0.1; histidine, 0.2; isoleucine, 0.4; leucine, 0.4; lysine, 0.4; methionine, 0.1; phenylalanine, 0.2; threonine, 0.4; tryptophan, 0.05; tyrosine, 0.2; valine, 0.4; and glutamine, 2.0. The complete composition of MEM is given in the preceding paper (11).

*2 The amino acid composition is as follows (in mM): Arginine, 0.03; cystine, 0.01; histidine, 0.01; isoleucine, 0.07; leucine, 0.04; lysine, 0.06; methionine, 0.002; phenylalanine, 0.03; threonine, 0.05; tryptophan, 0.005; tyrosine, 0.04; valine, 0.07; and glutamine, 1.0.
for 10 min. The supernatant solution (2 ml) was transferred to a counting vial with 15 ml of scintillation solution (I3). The TCA-insoluble precipitate was stood overnight with 5 ml of 90% formic acid to solubilize the proteins, and 2 ml of the resulting solution were placed in a vial with scintillation solution as above. Counts were measured in a Packard Tri-Carb liquid scintillation counter. Corrections for quenching were made from results on addition of standard isotope solution to test vials. All values are means of those in 2 flasks.

RESULTS

Effect of hydrocortisone on growth

The growth of cells cultured in MEM was depressed by addition of hydro-

![Graphs showing the effect of hydrocortisone on cell growth](image)

Fig. 1. Influence of hydrocortisone on the growth of HeLa cells cultured in media in which one amino acid was reduced so that growth was half that in MEM (Footnote *3). The deficient amino acid is indicated in the upper part of each figure. The levels of the deficient amino acids are given in Footnote,*2 and those of other amino acids are shown in Footnote.*1 Medium containing all amino acids at the levels shown in Footnote *1 is called MEM. "50% growth medium" means medium in which all amino acids are reduced simultaneously to give half the growth observed in MEM. The amino acid composition of "50% growth medium" is given in Footnote.*2 "Excess Ileu Med" means that isoleucine was added at 5 times the level of MEM. "Low Branched Chain Amino Acid" means that valine, leucine and isoleucine were reduced to 50% growth level at the same time. The numbers on the right of each graph show the incubation time (in days). Number of cells illustrated was measured microscopically by the method mentioned previously (7).

*3 The contents of all amino acids except one are the same as in MEM described in Footnote.*1 The amount of a reduced amino acids is the same level as described in Footnote,*3 e.g. methionine, 0.002 mM.
cortisone (Fig. 1), and similar growth inhibition was observed in 50% growth medium in which the concentrations of all amino acids were lowered so that growth was about one half of that in MEM. A low hydrocortisone level (0.02 μg/ml) effectively inhibited cell growth. Growth was also inhibited by addition of hydrocortisone to medium containing excess isoleucine in which the isoleucine content was 5 times that in MEM. Next hydrocortisone was added to media in which single amino acids were reduced to give 50% of the growth observed in MEM (II), while the levels of the other amino acids were the same as those in MEM. In media with reduced levels of isoleucine, methionine, or phenylalanine, addition of hydrocortisone caused increased growth. In medium with reduced levels of three branched chain amino acids, addition of hydrocortisone of 0.02 μg level increased growth a little, while addition of 2 μg of hydrocortisone strongly inhibited growth. In media with reduced histidine, or lysine, cell growth was not affected by addition of hydrocortisone at any levels tested. In low arginine medium, cell growth was inhibited by addition of 2 μg of hydrocortisone.

**Effect of hydrocortisone on the free amino acid contents of cells**

Endo et al. (1) reported a decrease in the free amino acid pool in HeLa cells cultured in MEM with hydrocortisone. Accordingly the effects of hydrocortisone on the free amino acids in cells cultured in media of low isoleucine or methionine content (where growth was increased by hormone addition) and low lysine content (where growth was unaffected by hormone treatment) were investigated. In

| Amino acid      | Low Met Med | Hydrocortisone (μmoles/mg nitrogen) |
|-----------------|-------------|------------------------------------|
| Aspartic acid   | 0.320       | 0.286                              |
| Glutamic acid   | 0.126       | 0.188                              |
| Glycine         | 0.042       | 0.044                              |
| Alanine         | 0.019       | 0.036                              |
| Valine          | 0.028       | 0.034                              |
| Cystine         | 0.032       | 0.028                              |
| Methionine      | —           | 0.002                              |
| Isoleucine      | 0.023       | 0.029                              |
| Leucine         | 0.036       | 0.038                              |
| Tyrosine        | 0.020       | 0.027                              |
| Phenylalanine   | 0.020       | 0.014                              |
| Histidine       | 0.015       | 0.020                              |
| Threonine + Serine | 0.044   | 0.038                              |
media of low isoleucine or methionine content, addition of hydrocortisone increased the level of each limiting amino acid. For example, in Low Met Med, free Met in the cells became detectable on addition of hydrocortisone. Only the case of Low Met Med is shown in Table 1. Effects of hydrocortisone on the contents of other amino acids which were not rate-limiting varied: some increased, some decreased, and some remained unchanged. In medium of low lysine content, addition of hydrocortisone did not affect the intracellular content of free lysine.

**Effect of hydrocortisone on incorporation of $^{35}$S-Met**

The time course experiment was planned to see whether the level of elevated free Met by hydrocortisone was maintained by accelerated transport of Met from Low Met Med to cells, or accumulated Met came from increased breakdown of cellular proteins induced by hydrocortisone. The time course of incorporation of DL-methionine-$^{35}$S into cells cultured in Low Met Med was examined with and without hydrocortisone added. Addition of hydrocortisone increased the incorporation of Met into the acid-soluble fraction at all times examined, and incorporation into the acid-insoluble fraction after incubation for 3 hr. Its addition caused about 20% increase in the incorporation of $^{35}$S-Met into the cells.

**Table 2. Change in the amount of labeled methionine incorporation into cells during culture in Low Met Med with or without hydrocortisone.** In experiments 1 and 2, the radioactivity of DL-methionine-$^{35}$S added to the culture media were 0.1 and 0.01 μCi, respectively per ml. Each value is the mean of two flasks.

| Time (hr) | 1  | 2  | 3  |
|----------|----|----|----|
| Exp. 1   |    |    |    |
| TCA-soluble | —  | 460| 587| 700|
|           | +Hydrocortisone | 490| 590| 885|
| TCA-insoluble | —  | 2,350| 5,840| 13,900|
|           | +Hydrocortisone | 2,850| 6,730| 16,440|
| Exp. 2   |    |    |    |
| TCA-soluble | —  | 23.2| 39.3| 34.1|
|           | +Hydrocortisone | 39.3| 49.6| 38.4|
| TCA-insoluble | —  | 575| 1,500| 2,540|
|           | +Hydrocortisone | 591| 1,310| 3,010|

**DISCUSSION**

**McCARL** and co-workers (3-4) reported that cortisol accelerated the beating of heart cells in vitro. **BALLARD** and **TOMKINS** (5) suggested that the synthesis of a specific cell surface factor was regulated by glucocorticoids. This indicates the possibility that glucocorticoids may alter the cell surface. We found that under some amino acid balances such as Low Met Med, hydrocortisone increased
the incorporation of amino acid into HeLa cells. This suggests that hydrocortisone may stimulate the transport of amino acids through the cell surface. This may be one of the mechanisms of the growth promoting effect of hydrocortisone on cells cultured in media in which amino acid compositions are unfavourable for cell growth.

Rancourt and Litwack (14) in electron microscopic study, observed changes of the rough surfaced endoplasmic reticulum induced by glucocorticoids in vivo. It is also possible that glucocorticoids affect protein synthesis in the cells, since Drews and Brawerman (15) reported that RNA obtained from cortisol-induced rats did not compete with that obtained from untreated rats in formation of hybrids with DNA. We observed morphological changes such as formation of giant cells and polynuclear cells, on treatment of cells with higher concentration of hydrocortisone. Previously we reported (11) that, generally speaking, the rate limiting factor for cell growth in media of various amino acid compositions was the amount of the most limiting amino acid. In the present work, a change of the growth rate of cells was observed on addition of hydrocortisone to the medium. Addition of hydrocortisone depressed growth in some media such as MEM, which has a balanced amino acid composition, but increased growth in some media such as Low Met Med, which has an unbalanced amino acid composition. Moreover, in medium deficient in three branched chain amino acids such as valine, leucine and isoleucine, hydrocortisone increased growth at low concentration and inhibited it at high concentration. The reasons for these complexities in the effects of hydrocortisone are unknown. Hydrocortisone may be effective at certain level to concentrate some amino acids into cells.

Our experiments have not yet clarified the mechanisms of the growth promotion effect of hydrocortisone. But it may be said that the effect of glucocorticoids in promoting growth of rats on an amino acid imbalanced diet may partly be due to its direct action.

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