# THE SPECIFIC AMINO ACID REQUIREMENTS OF A HUMAN CARCINOMA CELL (STRAIN HeLa) IN TISSUE CULTURE

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PLATES 5 TO 8

A previous paper from this laboratory (1) has described the specific amino acids required for the growth of a mouse fibroblast ("L" strain) in tissue culture. Those requirements, in addition to the nine amino acids known to be essential for the intact animal (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine), included arginine, cyst(e)ine, and tyrosine, all in the L configuration. The maximally effective concentrations ranged from 0.005 $\mu$M per ml. in the case of tryptophan, to 0.1 to 0.2 $\mu$M per ml. for isoleucine.

The present paper describes the amino acid requirements of a human uterine epithelial carcinoma cell (strain HeLa) cultured by Gey (2). As will be shown, despite the difference in animal species and in cell type, the amino acid requirements of the two cell lines were qualitatively similar. The quantitative differences are described in the text. Following papers will consider the vitamin requirements of the HeLa cell, the roles of glutamine and glutamic acid, and the need for serum protein.

## Methods and Materials

*Maintenance of Stock Cultures.*—The HeLa cell has generally been cultured in media containing 25 to 40 per cent human serum in balanced salt solution, sometimes supplemented with embryonic tissue extracts or ultrafiltrates. The basal medium described in Table I, based on previous studies with the "L" strain of mouse fibroblast (1), gave sustained growth when supplemented with 10 per cent human serum, and even better growth when the concentrations of the amino acids were appropriately modified in the light of the experiments to be here described. Stock cultures were maintained in 1 liter Blake bottles, in which the cells adhered to the glass and were overlaid with 50 to 60 ml. of the culture medium. When the cultures were ready for division the supernatant fluid was withdrawn, and the cells were overlaid with 10 ml. of fresh, warm medium at pH 7.8 to 8.0 containing 0.25 per cent Difco "1-250" bacto-trypsin. Within a few minutes, with rocking, the cell sheet began to come off the glass. The resulting cell aggregates were gently broken up with a pipette and centrifuged at 500 R.P.M. for 5 minutes. The sediment was then resuspended in fresh medium, sometimes without washing and sometimes after a single washing in fresh medium, and distributed into

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two to five times the original number of flasks, depending on the cell population. In these daughter cultures, the cells adhered to the glass within a few hours, and the rounded cells then flattened out within 18 to 30 hours to form small islands of typical flattened epithelial cells. The supernatant fluid was changed after 48 hours and at 2 to 3 day intervals thereafter, until the cultures were again ready for division, usually within 5 to 7 days.

*Preparation of Experimental Flasks.*—To determine the essentiality of the various growth factors under test, the cells were harvested from the Blake bottles in the usual manner with trypsin, and diluted in the stock serum–amino acid–vitamin mixture, to a final concentration of 60,000 to 200,000 cells per ml. 2 ml. of this mixture was inoculated into flasks modified from the T15 flasks described by Earle and Highhouse (4) by placing the neck on the triangular rather than on the flat end of the flask. 24 hours later, after the cells had adhered

TABLE I

*Basal Medium* Used for the Identification of the Amino Acid Requirements of the HeLa Cell*

| Amino acids‡ | | Vitamins | | Inorganic salts§ | | Additional growth factors | |
|---|---|---|---|---|---|---|---|
| | *mM* | | *gm. per ml.* | | *per cent* | | |
| Arginine | 0.1 | *p*-Aminobenzoic acid | $10^{-7}$ | NaCl | 0.68 | Glucose | 0.1 per cent |
| Cyst(e)ine | 0.05 | Biotin | $10^{-6}$ | KCl | 0.04 | Glutamine | 1 mM |
| Histidine | 0.02 | Choline | $10^{-4}$ | NaH$_2$PO$_4$·H$_2$O | 0.014 | | |
| Isoleucine | 0.2 | Folic acid | $10^{-6}$ | NaHCO$_3$ | 0.22 | | |
| Leucine | 0.1 | Nicotinic acid | $10^{-6}$ | CaCl$_2$ | 0.02 | | |
| Lysine | 0.1 | Nicotinamide | $10^{-6}$ | MgCl$_2$ | 0.0076 | Antibiotics and pH indicator | |
| Methionine | 0.05 | Pantothenic acid | $10^{-6}$ | | | | |
| Phenylalanine | 0.05 | Pyridoxal | $10^{-6}$ | Fe(NO$_3$)$_3$ | 0.00001 | Penicillin | 50 μg./ml. |
| Threonine | 0.1 | Pyridoxine | $10^{-6}$ | | | Streptomycin | 50 μg./ml. |
| Tryptophan | 0.01 | Riboflavin | $10^{-7}$ | | | Phenol red | 5 μg./ml. |
| Tyrosine | 0.10 | Thiamin | $10^{-6}$ | | | | |
| Valine | 0.1 | | | | | | |

* Supplemented with 10 per cent whole human serum or 5 per cent dialyzed human serum (*cf.* text).
‡ All in L-configuration.
§ In concentrations present in Earle's balanced salt solution (3).

to the glass, the supernatant fluid was withdrawn and replaced with the various experimental media under test.

*Evaluation of Growth Response.*—The experimental fluid was replaced daily until the cultures were ready for enumeration; *i.e.*, when the control flasks had grown out to form a solid sheet covering the bottom of the flask.

In order to obtain reasonably dispersed and adequately stained suspensions, it was found necessary to modify the method of Sanford *et al.* (5) in the following manner. The fluid was withdrawn, and the cell layer was overlaid for one hour with 1.8 ml. of a staining solution containing 0.2 M citric acid and 0.1 per cent crystal violet. The flasks were then shaken for 10 minutes in a mechanical shaker at approximately 300 oscillations per minute, after which 0.2 ml. of 0.5 M Na$_2$HPO$_4$ was added to each flask. The cell nuclei were then ready for immediate counting in a hemocytometer chamber.

The experiments here described would not have been possible without the expert and valued assistance of Mr. Vance I. Oyama.

## TABLE II

*Growth Response of the HeLa Cell to Individual Amino Acids as a Function of Their Concentration in the Medium*

| Amino acid studied | Inoculum × 10⁴ | Duration of incubation | Concentration of specific amino acid, $\mu M$ per ml. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 10 | 3 | 1 | 0.3 | 0.3 | 0.03 | 0.01 | 0.003 | 0.001 | 0 |
| | | | Degree of cellular multiplication, referred to inoculum as 1 | | | | | | | | | |
| | | *days* | | | | | | | | | | |
| L-Arginine | 44 | 5 | 0.5 | 4.8 | 4.7 | 5.0 | 5.6 | 2.7 | 1.6 | | | 0.7 |
| | 30 | 5 | | | | | 4.4 | 3.3 | 1.7 | 1.1 | 0.8 | 0.8 |
| L-Cystine | 27 | 6 | | 1.6 | 2.5 | 3.7 | 4.7 | 4.5 | 3.3 | 0.4 | | 0.3 |
| L-Histidine | 44 | 5 | 4.6 | 4.1 | 5.3 | 5.1 | 4.3 | 4.9 | 1.9 | | | 0.5 |
| | 30 | 5 | | | | | 6.5 | 6.4 | 3.4 | 1.7 | 0.8 | 0.6 |
| L-Isoleucine | 20 | 6 | 3.8 | 6.5 | 6.4 | 7.2 | 8.5 | 2.7 | 0.8 | 0.3 | | <0.1 |
| L-Leucine | 20 | 6 | 3.0 | 5.9 | 8.1 | 7.0 | 6.1 | 4.7 | 2.7 | 2.5 | | 1.6* |
| | 30 | 5 | | | 4.8 | 5.1 | 5.9 | 3.8 | 2.4 | 2.0 | 1.6 | 1.3* |
| L-Lysine | 44 | 5 | 0.007 | 0.5 | 4.8 | 4.8 | 4.4 | 1.1 | 0.5 | | | 0.3 |
| | | | | | | | | | | | | 0.5 |
| L-Methionine | 20 | 6 | | 6.6 | 8.0 | 8.9 | 8.2 | 8.5 | 2.6 | 1.0 | | 0.4 |
| | 30 | 5 | | | | | 6.9 | 6.0 | 2.2 | | | 0.4 |
| L-Phenyl-alanine | 20 | 6 | 4.4 | 7.5 | 9.3 | 7.3 | 10.5 | 7.2 | 1.6 | 0.8 | | 0.3 |
| | 30 | 5 | | | | | 6.8 | 4.3 | | | | 0.5 |
| L-Threonine | 20 | 6 | 6.2 | 6.9 | 9.5 | 4.5 | 5.4 | 2.9 | 0.9 | 0.3 | | 0.2 |
| L-Tryptophan | 20 | 7 | | | 0.2 | 3.9 | 9.9 | 10.3 | 7.3 | 4.3 | | 2.2‡ |
| | 30 | 5 | | | | | | 5.7 | 5.6 | 4.1 | 2.4 | 2.0‡ |
| L-Tyrosine | 27 | 6 | | 3.5 | 4.3 | 4.0 | 4.6 | 6.0 | 1.6 | 0.7 | | 0.3 |
| L-Valine | 27 | 6 | 3.1 | 3.2 | 4.1 | 5.2 | 4.9 | 2.6 | 0.7 | 0.1 | | <0.1 |

* The slight multiplication in the supposed absence of L-leucine was due to contamination of some of the amino acids with small amounts of this compound, and was not observed when enzymatically resolved amino acids were used (*cf.* Table IV).

‡ This initial multiplication in the absence of L-tryptophan was followed by the degeneration and death of the cells (*cf.* Table IV).

## EXPERIMENTAL

*The Amino Acids Essential for the Growth of the HeLa Cell.*—In order to determine whether the amino acids essential for the growth of mouse fibro-

blasts were similarly essential for the HeLa cell, the basal medium of Table I was supplemented with 5 per cent of dialyzed human serum instead of 10 per cent whole serum, as in the stock cultures. In such a medium, cells continued to grow and multiply, and the scattered islands of the inoculum coalesced to form continuous sheets of piled up cells. This is indicated in Figs. 1
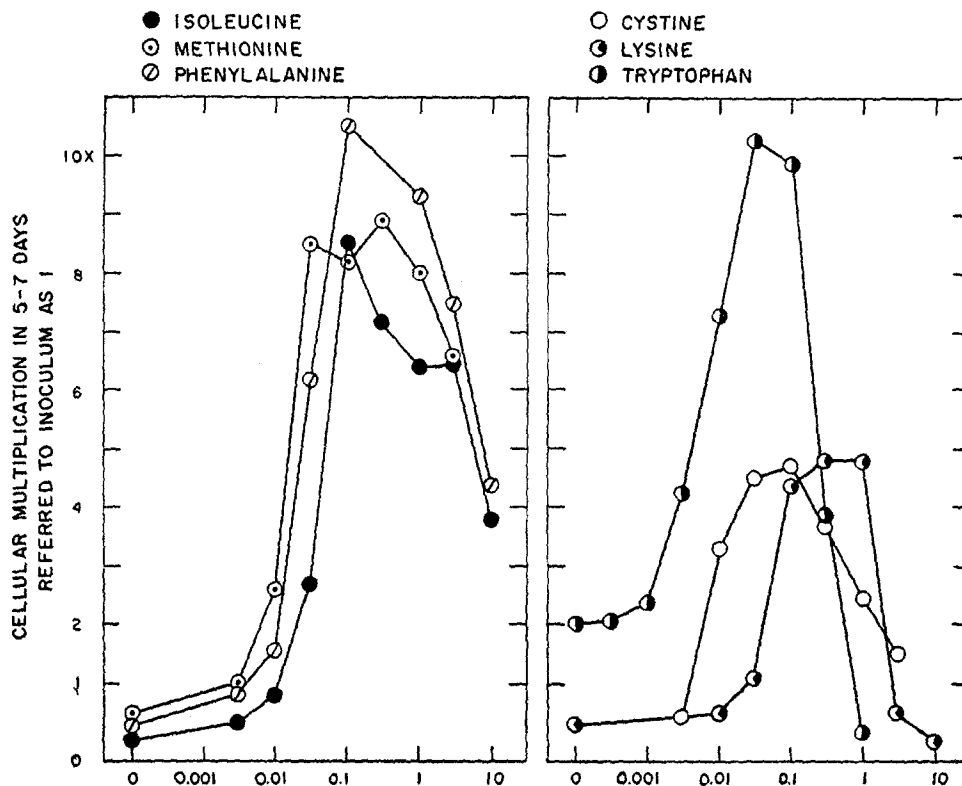


TEXT-FIG. 1. The growth response of the HeLa cell in 5 to 7 days to varying concentrations of a number of essential amino acids. Showing the inhibition often observed at concentrations in excess of an optimal level.

to 4. However, when a single essential amino acid was omitted from the medium, multiplication stopped. Within 1 to 4 days there were obvious degenerative changes in the cells, and the culture eventually died. This progressive deterioration is illustrated for cells in a phenylalanine-deficient medium in Figs. 5 to 7; and the degenerative changes produced within 3 to 7 days by six amino acid deficiencies are shown in Figs. 8 to 13. In this manner, ten of the twelve amino acids listed in Table I (arginine, cyst(e)ine, histidine, isoleucine, ly-

sine, methionine, phenylalanine, threonine, tyrosine, and valine) were shown to be immediately essential for the growth of the HeLa cell.

TABLE III

*The Failure of the D-Enantiomorphs of the Essential Amino Acids to Support the Growth of the HeLa Cell, or to Inhibit the Growth Response to the L-Isomer*

| Amino acid | Concentration in medium of | | Growth response* in 6 to 8 days | Amino acid | Concentration in medium of | | Growth response* in 6 to 8 days |
|---|---|---|---|---|---|---|---|
| | D-, mM | L-, mM | | | D-, mM | L-, mM | |
| Arginine | 1 | — | 1.0 | Phenylalanine | — | — | 0.8 |
| | 0.1 | — | 0.9 | | 0.1 | — | 0.6 |
| | — | 0.1 | 5.6 | | — | 0.1 | 6.8 |
| | | | | | 1 | 0.1 | 5.2 |
| Histidine | 1 | — | 0.4 | Threonine | 1 | — | 1.2 |
| | 0.1 | — | 0.5 | | 0.1 | — | 0.2 |
| | — | 0.1 | 4.3 | | — | 0.1 | 5.4 |
| Isoleucine | 1 | — | 0.4 | Tryptophan | 0.5 | — | 1.7 |
| | 0.1 | — | <0.1 | | 0.05 | — | 2.5 |
| | — | 0.1 | <0.1 | | — | 0.05 | 5.7 |
| | | | | | 0.5 | 0.05 | 5.2 |
| Leucine | 1 | — | 1.5 | Tyrosine | 1.0 | — | <0.1 |
| | 0.1 | — | 1.6 | | 0.1 | — | <0.1 |
| | — | 0.1 | 5.9 | | — | 0.1 | 4.6 |
| | 1 | 0.1 | 6.0 | | | | |
| Lysine | 1 | — | 0.8 | Valine | 1 | — | 0.1 |
| | 0.1 | — | 0.5 | | 0.1 | — | 0.1 |
| | — | 0.1 | 5.6 | | — | 0.1 | 4.9 |
| Methionine | 1 | — | 0.7 | | | | |
| | 0.1 | — | 0.7 | | | | |
| | — | 0.1 | 6.9 | | | | |
| | 1 | 0.1 | 6.8 | | | | |

* Degree of cellular multiplication, referred to inoculum as 1.

In the absence of tryptophan, there was moderate initial growth, but the cultures degenerated after 7 to 10 days and could not then be successfully divided (*cf.* Table IV); while the heavy growth occasionally observed in the absence of leucine proved to be due to contamination of several of the other amino acids with this compound. On the use of enzymatically resolved L-methionine, L-cystine, L-tyrosine, and L-isoleucine instead of the commerical

TABLE IV

*The Progressive Dissolution of HeLa Cells in the Absence of a Single Essential Amino Acid, and Their Recovery and Remultiplication on the Addition of the Missing Component*

| Specific amino acid omitted from medium | No. days in deficient medium | Cell nuclei count* | Cell count after indicated no. days in "revival" medium | | | |
|---|---|---|---|---|---|---|
| | | | 2 | 4 | 6 | 7 |
| Control (complete medium) | 0 | 1 | 1.8‡ 2.0‡ | 4.9 4.9 | 11.8 10.0 | |
| L-Histidine | 3 | 1.25 | 1.2 | 2.2 | | 6.0 |
| | 5 | 0.9 | | | | |
| | 10 | 0.8 | | | | |
| L-Leucine§ | 3 | 0.7 | 1.0 | 1.9 | 2.5 | |
| | 5 | 0.15 | | | | |
| | 6 | 0.2 | 0.05 (3 days) | | < | |
| L-Lysine | 3 | 0.7 | 1.2 | 3.5 | | 9.8 |
| | 7 | 0.7 | | | | 5.4 |
| | 10 | 0.5 | | 2.6 | | |
| L-Phenylalanine | 3 | 1.1 | 1.3 | 3.8 | | 6.6 |
| | 5 | 1.0 | | | | |
| | 7 | 0.5 | 0.7 | | | |
| L-Tryptophan | 3 | 1.5 | | | | |
| | 5 | 3.8 | | | | |
| | 6 | 3.2 | 4.4 | | 5.4 | |
| | 9 | 2.6 | 5.4 (3 days) | | | |
| | 12 | 2.2 | | | | |
| L-Tyrosine | 3 | 1.2 | 1.6 | 4.6 | | 14.6 |
| | 5 | 1.3 | | | | |
| | 7 | 0.6 | 1.3 | 6.4 | | |

\* Referred to count at time 0 as 1. Each number in the body of the table is the average of 3 to 4 replicate flasks.

‡ Results obtained in 2 different experiments, carried out at different times.

§ Rapidly progressing evidence of leucine deficiency in this experiment, using enzymatically resolved L-cystine, L-tyrosine, and L-isoleucine in the basal medium is to be contrasted with the slow multiplication observed in the supposed absence of leucine when L-amino acids of commerce were used instead (cf. Table II and page 41).

samples, L-leucine was found to be rigorously essential for growth under the conditions of the present experiments (cf. Table IV). The courtesy of Dr. Jesse L. Greenstein in making these L-amino acids available is gratefully acknowledged.

*Quantitative Requirements of the 12 Essential Amino Acids.*—In media containing varying amounts of a single essential amino acid, with all the others in adequate and fixed concentrations, the amount of growth increased with the concentration of the compound to reach a maximum at a concentration which varied from 0.01 mM in the case of tryptophan to 0.1 mM in the case of most of the other amino acids. This growth response curve is illustrated for histidine in Figs. 14 to 17, and for cystine in Figs. 18 to 23. The results for all the compounds are summarized in Table II and Text-fig. 1. With some
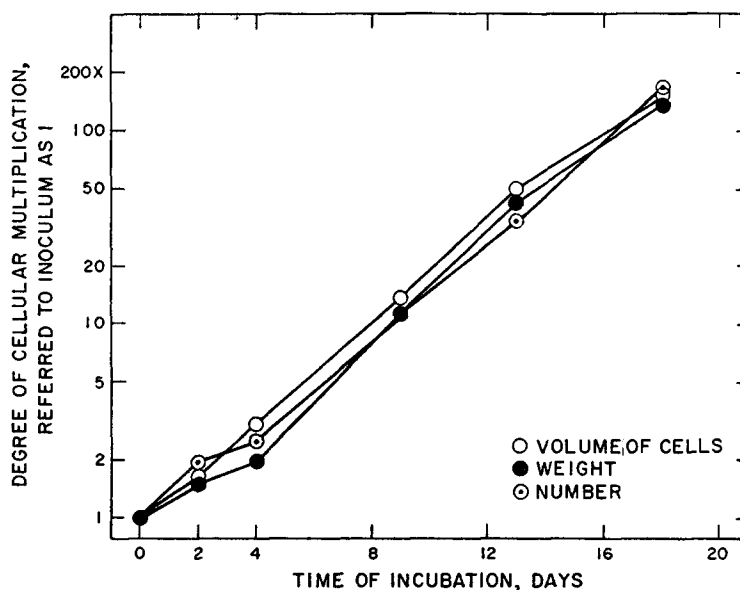


Text-Fig. 2. The progressive multiplication of the HeLa cell in "optimal" synthetic medium supplemented with 5 per cent of dialyzed human serum. The component amino acids were used in twice the maximally effective concentrations listed in Table V. The other factors were used at the concentrations shown in Table I.

of the amino acids, concentrations 10 to 100 times the maximally effective level did not further affect the growth of the cell. With some of the compounds, however (*cf.* isoleucine, methionine and phenylalanine in left-hand portion of Text-fig. 1), concentrations of 10 mM caused a significant inhibition of growth, while in the case of cystine, lysine, and tryptophan, concentrations of 10, 3, and 1 mM, respectively, caused a 90 per cent inhibition.

*Activity of the* D-*Enantiomorphs.*—As with the mouse fibroblasts, none of the D-amino acids proved capable of supporting the growth of the HeLa carcinoma cell (Table III). With D- instead of L-amino acids in the medium, the cells degenerated and died as if that amino acid had been omitted. However, the D-amino acid was not actively inhibitory. When a 10- to 100-fold excess of

the D-amino acid was added to optimal concentrations of the L-amino acid, there was the same amount of growth as if the medium contained the L-amino acid alone. This is illustrated for D- and L-lysine in Figs. 24 to 27.

*Early Reversibility of the Cytopathogenic Effects of Amino Acid Deficiencies.*— The profound microscopic changes caused by single amino acid deficiencies, illustrated in Figs. 5 to 7 and Figs. 8 to 13, eventually led to the death and dissolution of the cell. In their early stages, however, these changes were
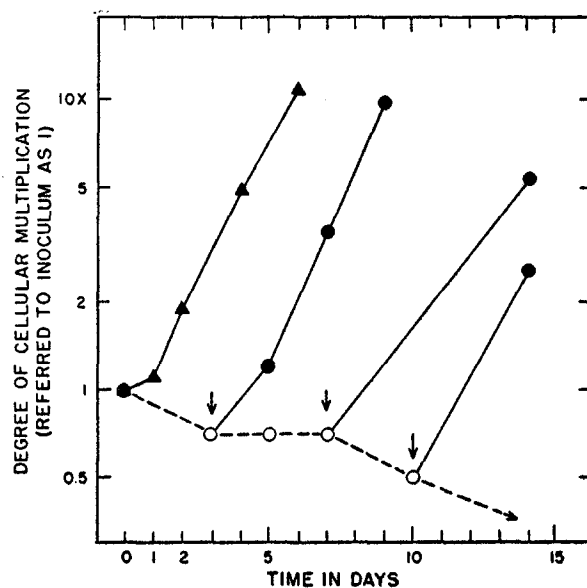


TEXT-FIG. 3. The revival of lysine-deficient HeLa cells by the restoration (↓) of the missing amino acid.

▲-▲, growth of HeLa cells in a control complete medium.

O-O, cells degenerating in the absence of lysine.

●-●, revival and remultiplication on the addition of lysine.

reversible. If cells were allowed to degenerate for 1 to 4 days in a medium lacking an essential amino acid, and if the missing compound was then restored, the cells regained their normal appearance in 1 to 3 days, and resumed multiplication at the usual rate (Table IV).

This revival and remultiplication is illustrated for histidine-deficient and phenylalanine-deficient cells in Figs. 28 to 31 and 32 to 34. The results with a number of amino acids are summarized in Table IV. The time for which the amino acid–deficient cells remained viable, *i.e.* capable of recovery on the addition of the missing compound, and the rate of that recovery process, varied widely among the individual amino acids. With some, as illustrated for

L-lysine in Text-fig. 3, many of the markedly degenerated cells remained viable, and could be resuscitated after as long as 7 days in the deficient medium, with little or no lag before the cells resumed multiplication. With other amino acids, however, the recovery process was much slower; and in some instances, the degenerated cell could not be revived even after 3 to 4 days in the deficient medium. These differences could reflect either the varying degree to which the individual amino acids are present as trace contaminants in the other compounds, the varying reserve of each in the cellular amino acid pool, or their varying rate of utilization.

## DISCUSSION

At least 12 amino acids have thus proved essential for the growth of a human carcinoma cell under the conditions of the present experiments. Those 12 include all 8 amino acids essential for the nutrition of man (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) and in addition, arginine, cyst(e)ine, tyrosine, and histidine. It may be that these cells are unable to make these 4 amino acids from their normal precursors. In such case, on a diet limited to the 8 amino acids essential for nitrogen balance in man, these 4 would presumably be provided either by the breakdown of other body cells, or by their overproduction in some other tissue. These cells would thus be parasitic with respect to some of their amino acid requirements. It is, however, conceivable that the HeLa cell does in fact synthesize small amounts of these 4 amino acids, sufficient for their relatively slow rate of growth *in vivo*, but inadequate for their rapid growth under the conditions of the present experiments. These possibilities are under present study.

In the first two columns of Table V are shown the concentrations of the various amino acids necessary for optimal growth of the HeLa cell under the conditions of the present experiments, compared with the concentration of those amino acids present in human serum. Despite the approximate nature of the determinations of the requirements for optimum growth (Table II), the parallelism in these values is self-evident and probably significant. The corresponding data for the mouse fibroblast and mouse serum (or plasma) are also included in Table V. The close parallelism between the concentration of the several amino acids optimal for the HeLa cell and for the mouse fibroblast is evident in columns 2 and 4. In most instances the HeLa cell required twice as much of each amino acid. The correlation between the amino acid requirements of the mouse fibroblast, and their concentrations in mouse serum (*cf.* columns 4 and 5 of Table V) is not as satisfactory as in the case of the HeLa cell and human serum.

In a medium containing the optimal concentrations of each of the 12 essential amino acids, but deficient in alanine, aspartic acid, proline, hydroxyproline,

and serine,[1] growth continued at an unchanged rate over a period of 18 days. During this time the cultures were divided four times and there was a 150-fold growth, whether measured by cell volume, cell mass, or cell number (cf. Text-fig. 2). The generation time in this experiment thus averaged 2.5 days. It is unlikely that these five compounds were essential, but were being supplied by the breakdown of the serum protein present in the medium. In that event, one would reasonably expect that the protein would also have supplied some of the 12 demonstrably essential amino acids, several of which sufficed to main-

TABLE V

*The Amino Acid Requirements for the Optimum Growth of a Human Carcinoma Cell (Strain HeLa) and of a Mouse Fibroblast (Strain "L") in Vitro, Considered in Relation to the Serum Concentrations*

| Amino acid | Man | | Mouse | |
|---|---|---|---|---|
| | Optimum for growth of HeLa cell *in vitro* | Concentration in serum* | Optimum for growth of "L" fibroblast *in vitro* | Concentration in serum* |
| | μM/ml. | μM/ml. | μM/ml. | μM/ml. |
| Tryptophan | 0.01 | 0.054 | 0.005 | 0.1 |
| Histidine | 0.02 | 0.09 | 0.01 | 0.1 |
| Cystine | 0.03 | 0.058 | 0.01 | 0.033 |
| Tyrosine | 0.03 | 0.083 (0.046) | 0.02–0.05 | 0.14 |
| Methionine | 0.03 | 0.033 | 0.02–0.05 | 0.13 |
| Phenylalanine | 0.05 | 0.08 (0.05) | 0.02 | 0.15 |
| Arginine | 0.05 | 0.1 | 0.05 | 0.05 |
| Leucine | 0.1 | 0.11 | 0.05 | 0.18 |
| Threonine | 0.1 | 0.17 (0.12) | 0.05 | 0.29 |
| Valine | 0.1 | 0.24 | 0.05 | 0.37 |
| Lysine | 0.1 | 0.21 | 0.05 | 0.44 |
| Isoleucine | 0.1 | 0.12 (0.07) | 0.1–0.2 | 0.11 |

* After Albritton (6). Values given in parentheses are after the data of Stein and Moore (7), when their values differ significantly from the averages cited by Albritton.

tain growth when as little as 0.003 to 0.01 μM per ml. were added to the medium containing the serum protein. The failure of the cells to grow in a medium deficient in any one of these 12 compounds indicates that the protein was not being broken down sufficiently to provide either the essential amino acids, or materials which could be substituted for them. Further, as in the case of the mouse fibroblast (1), it is hardly a coincidence that every one of the 8 amino acids essential to man proved similarly essential to the HeLa cell under the conditions of the present experiment; while conversely, none of the 5 amino

[1] In these experiments, a potential source of glutamic acid was being supplied, as glutamine. The relative importance of these two compounds in the nutrition of the HeLa cell will be discussed in a following paper.

acids not necessary for the growth of the HeLa cell under the conditions of the present experiments are essential for growth in man. The tentative conclusion seems justified that these 5 amino acids are, in fact, not essential for the survival and multiplication of the HeLa cell, even on long-term propagation; *i.e.*, the cell can synthesize these compounds at a rate sufficient for its requirements.

As in the case of the mouse fibroblast (1), the technic used in these experiments makes it possible to produce microscopic changes corresponding to single amino acid deficiencies. These changes were completely reversible in their early stages. Further, as illustrated in Figs. 8 to 13, the gross appearance of the cells varied widely according to which amino acid had been withdrawn from the medium. Studies of these cellular changes as visualized in the phase and electron microscope are now in progress. It is perhaps significant that the cytopathogenic changes produced in these cells by specific amino acid deficiencies closely resemble those observed after their inoculation with a number of viral agents.

It deserves emphasis that, again corresponding to the observations with mouse fibroblasts, these human carcinoma cells can survive and multiply in a medium containing only amino acids, a limited number of vitamins, glucose, glutamine, balanced salt solution, and serum protein. The absence of added purines, pyrimidines, fat-soluble vitamins, or of tissue extractives other than serum protein, is particularly to be noted. These cells apparently can synthesize nucleic acids, proteins, and all the cofactors required in their metabolic activities, from these relatively simple materials. The nutritional role of the serum protein is under present study; but, as discussed above, it is probably not serving as a source of amino acids.

The optimal medium for the cultivation of the HeLa cell as defined by the present experiments includes each of the 12 essential amino acids at approximately twice the concentration found to suffice for maximal growth (*cf.* Table V), the vitamins, salts, and accessory growth factors listed in Table I, supplemented with serum protein. For purposes of routine cultivation, the latter may be supplied as 10 per cent whole human serum. The cell has now been continuously propagated in this medium for 6 months. The generation time in young cultures averages approximately 48 hours. As will be described in following papers, this medium has been successfully used for the direct isolation and propagation of an epithelial carcinoma of the jaw (strain KB). The degree to which the nutritional requirements of this and other cells of human origin differ in detail from those of the HeLa cell is under present study.

## SUMMARY

The amino acid requirements of a human uterine carcinoma cell (HeLa strain) have been defined. The 12 compounds previously found to be essential

for the growth of a mouse fibroblast proved similarly essential for this human epithelial cell. They included arginine, cyst(e)ine, histidine, and tyrosine, in addition to the eight amino acids required for nitrogen balance in man (iso-leucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). Only the L-amino acids were active; the D-enantiomorphs had no demonstrable effect at physiologic concentrations.

The minimum concentrations required for survival and limited growth varied from 0.003 μM per ml. for L-tryptophan, to 0.1 μM per ml. for L-lysine. The concentrations permitting optimum growth similarly varied from 0.01 μM per ml. for tryptophan, to 0.1 μM per ml. for leucine, isoleucine, threonine, lysine, and valine. The latter optimum concentrations of the individual amino acids were closely correlated with their serum levels. With at least six of the amino acids, high concentrations, in the range 1 to 10 μM per ml., caused a definite growth inhibition.

In the absence of a single essential amino acid, degenerative changes oc-curred in the cells, culminating in their death and dissolution. In the early stages, however, these degenerative changes could be reversed by the restora-tion of the missing component.

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## EXPLANATION OF PLATES

### PLATE 5

FIGS. 1 to 4. The multiplication of HeLa cells in an optimal medium (growth after 1, 2, 3, and 4 days in medium of Table I). × 110.

FIGS. 5 to 7. The progressive degeneration of HeLa cells in a phenylalanine-de-ficient medium (appearance of cells after 5, 7, and 10 days). × 115.
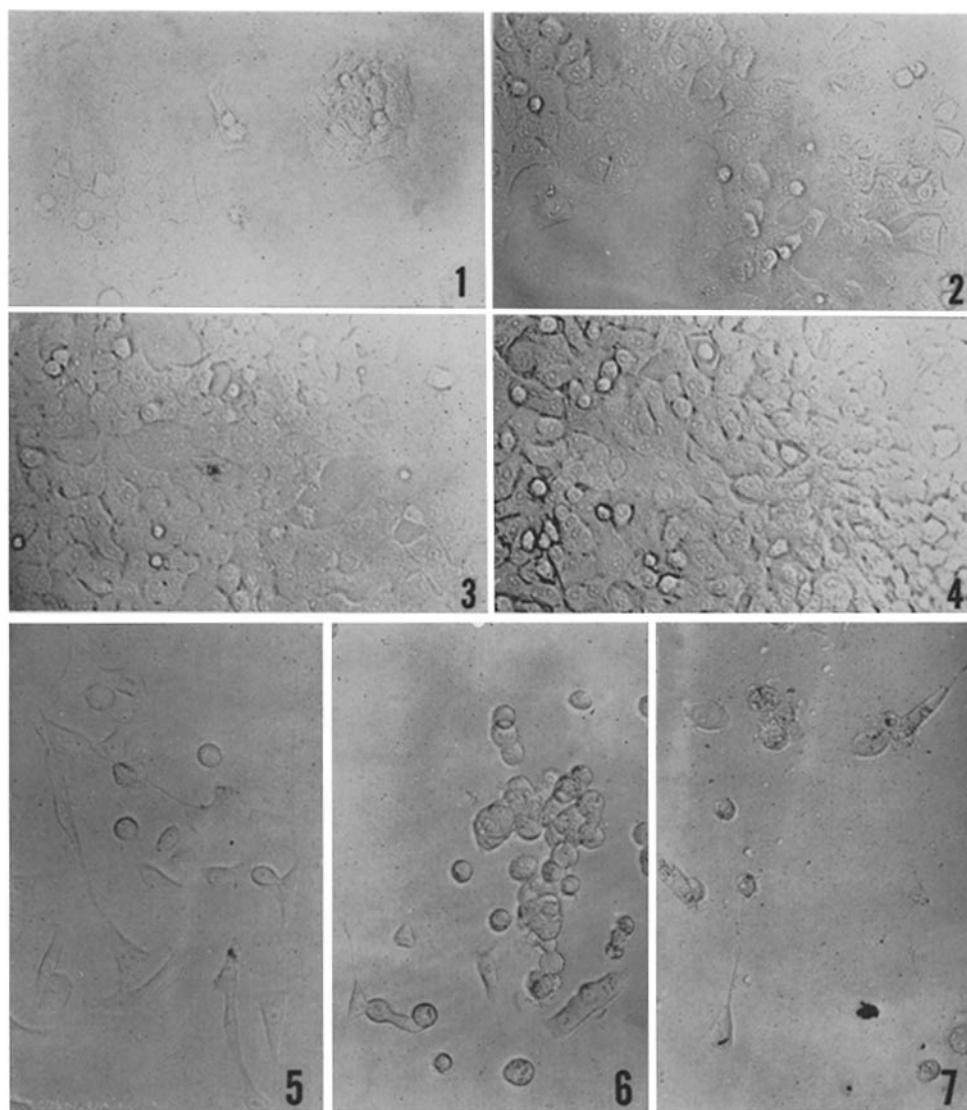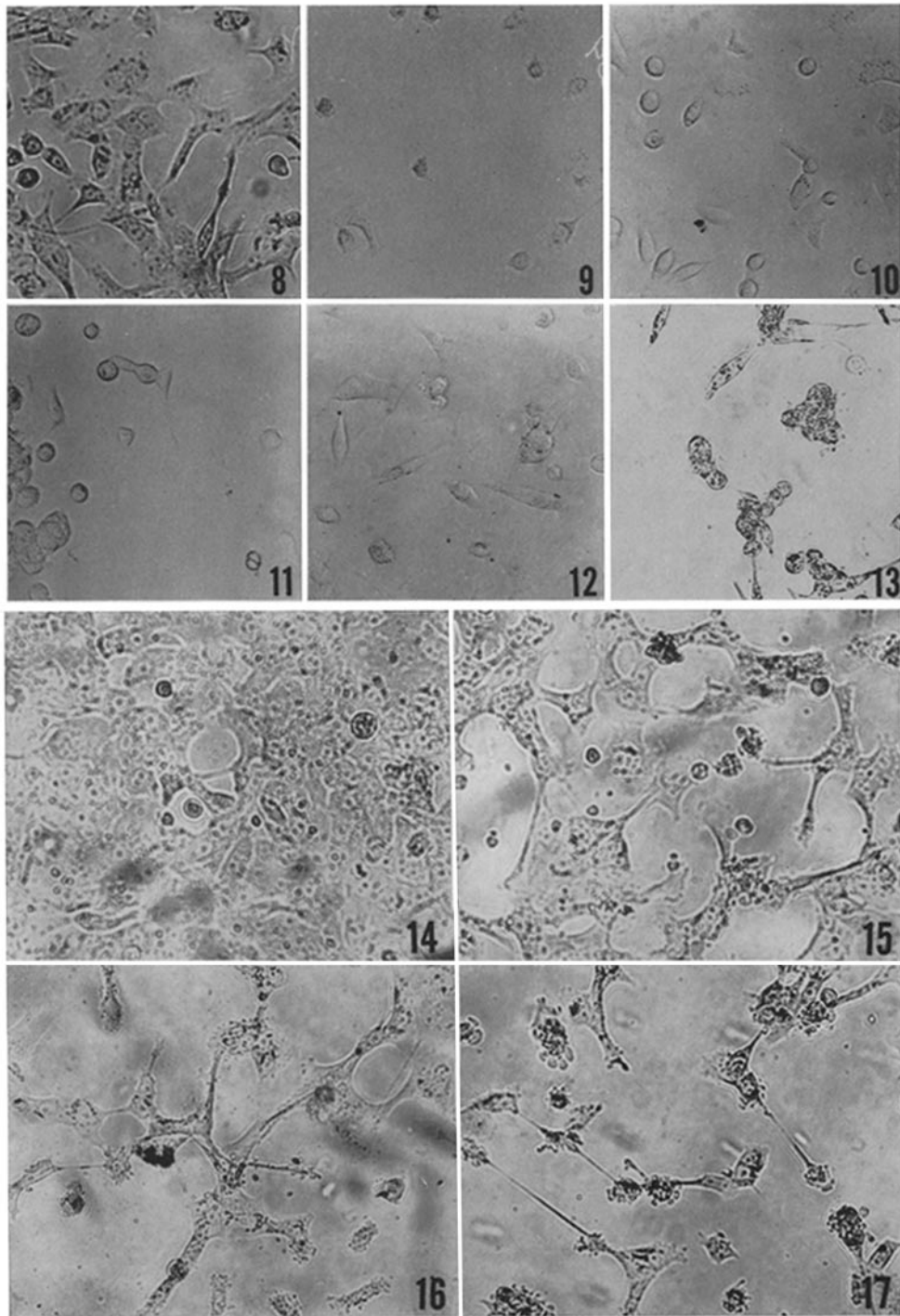
(Eagle: Amino acid requirements of human cancer cell)

FIGS. 8 to 13. The degenerative changes produced in HeLa cells by single amino acid deficiencies. × 120.

FIG. 8. Arginine deficiency (5 days). Fig. 9. Histidine deficiency (3 days). Fig. 10. Lysine deficiency (7 days). Fig. 11. Phenylalanine deficiency (7 days). Fig. 12. Tyrosine deficiency (7 days). Fig. 13. Cystine deficiency (6 days).

FIGS. 14 to 17. The 5 day growth response of the HeLa cell to varying concentrations of histidine (0.01, 0.003, 0.001, and 0 mM, respectively). × 130.

(Eagle: Amino acid requirements of human cancer cell)

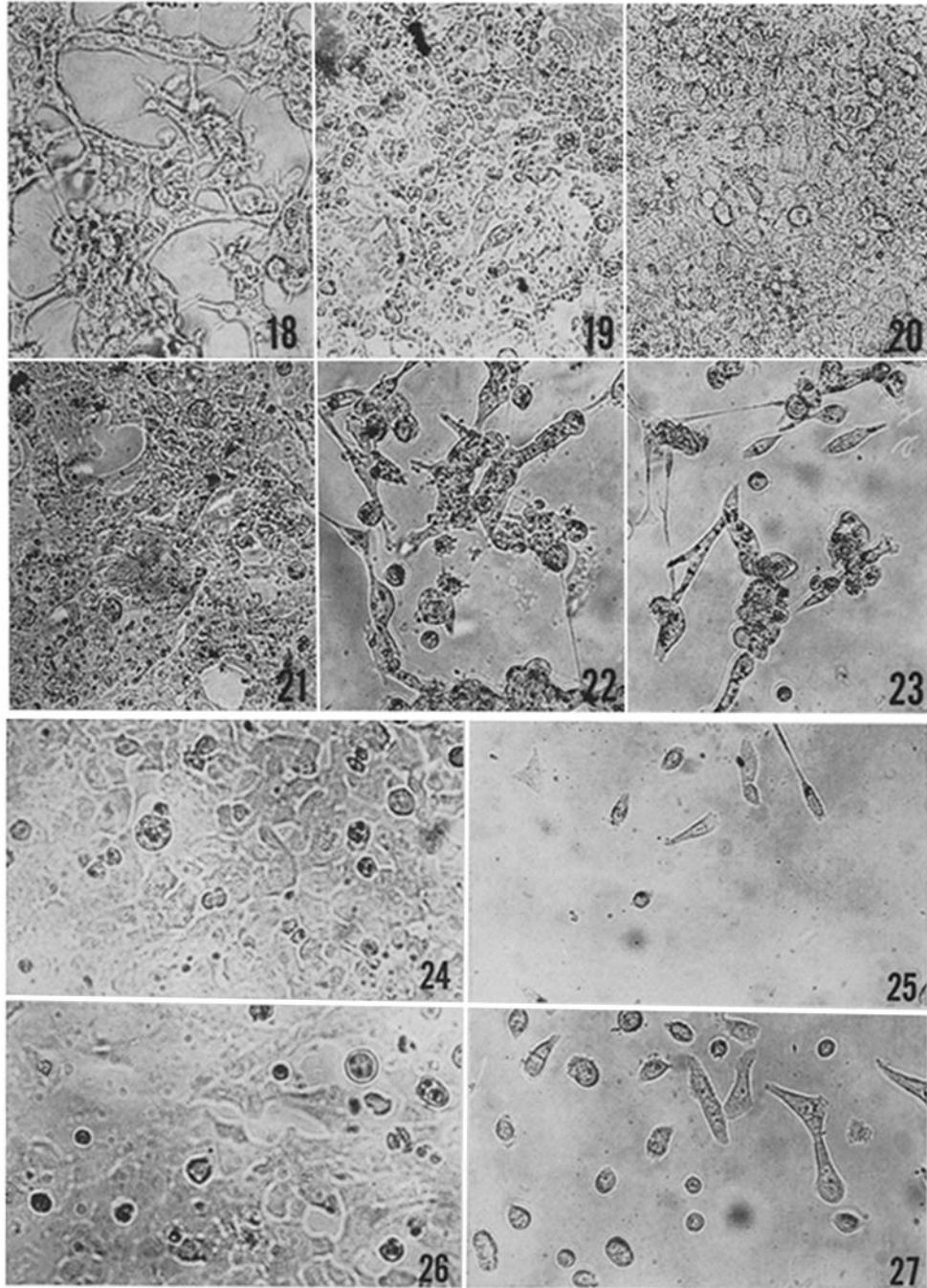FIGS. 18 to 23. The 5 day growth response of the HeLa cell to varying concentrations of L-cystine (3, 1, 0.1, 0.01, 0.003, and 0 mM, respectively). × 130.

FIGS. 24 to 27. The 5 day growth response of the HeLa cell to D- and L-lysine. × 110.

Fig. 24. L- at 0.1 mM. Fig. 25. D- at 0.1 mM. Fig. 26. D- at 1 mM. + L- at 0.1 mM. Fig. 27. 0

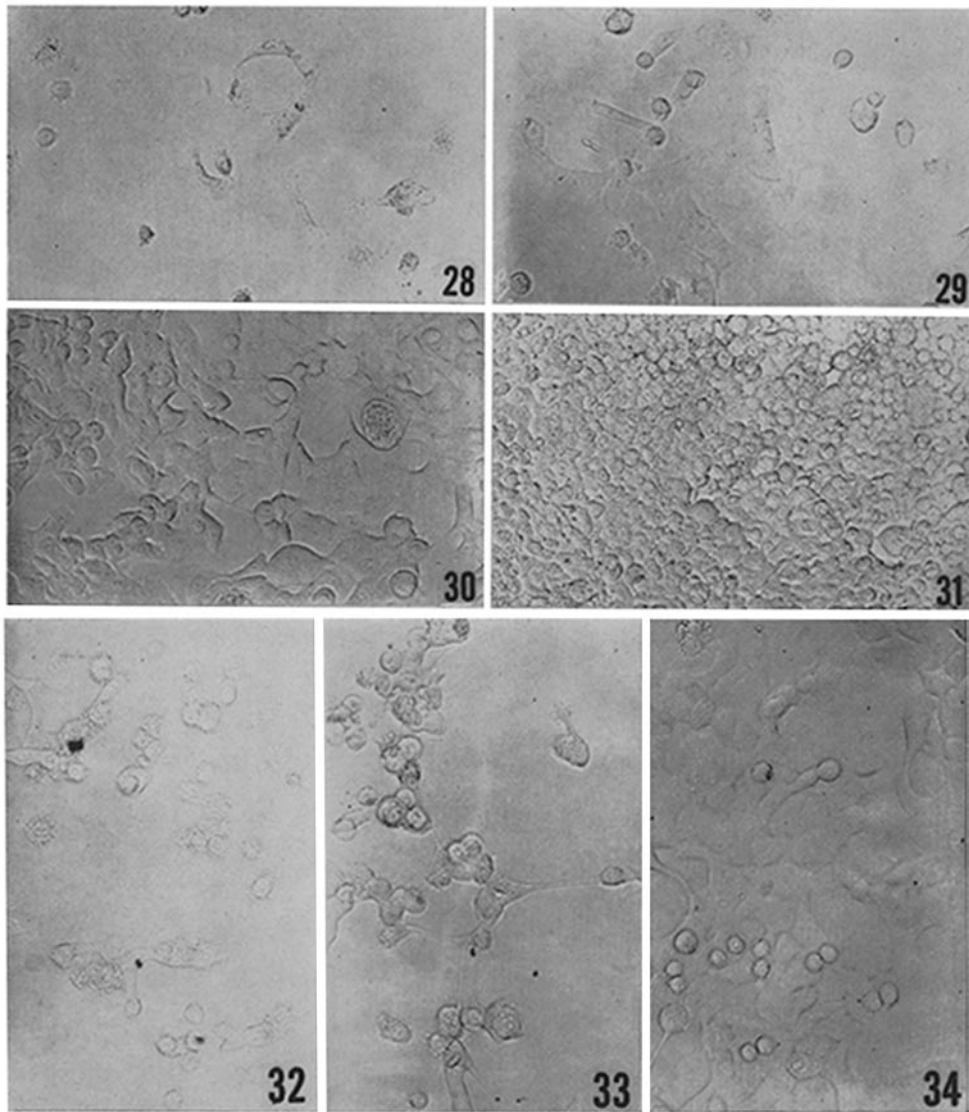(Eagle: Amino acid requirements of human cancer cell)

FIGS. 28 to 31. The recovery of HeLa cells from a histidine deficiency. × 112.

Fig. 28. Appearance of cells after 3 days in histidine-deficient medium. Fig. 29. 1 day after restoration of histidine to medium. Fig. 30. 4 days after restoration of histidine to medium. Fig. 31. 7 days after restoration of histidine to medium.

FIGS. 32 to 34. The recovery of HeLa cells from a phenylalanine deficiency. × 100.

Fig. 32. Appearance of cells after 3 days of phenylalanine-deficient medium. Fig. 33. 2 days after restoration of phenylalanine to medium. Fig. 34. 4 days after restoration of phenylalanine to medium.

(Eagle: Amino acid requirements of human cancer cell)