STUDIES ON CONTACT INHIBITION OF GROWTH IN THE MOUSE FIBROBLAST, 3T3

II. EFFECTS OF AMINO ACID DEPRIVATION AND SERUM ON GROWTH RATE

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SUMMARY

The effect of amino acid concentration on the initial growth rate of the contact-inhibited mouse fibroblasts 3T3 and its SV40-transformed derivative 3T3T has been studied. In medium in which the serum had been passed through a Sephadex column, and the concentration of amino acids lowered 1000-fold, one acid at a time, the growth rate constants were computed. For most of the 16 amino acids studied, the initial growth rate of 3T3 decreased more profoundly than that of 3T3T. The presence in serum of a factor which stimulates amino acid uptake and growth rate of 3T3T is described.

INTRODUCTION

In a previous paper (Meisler, 1973) it has been shown that the established mouse embryo cell line 3T3, and to a lesser degree the 3T3 transformed by SV40 (3T3T), exhibit unbalanced growth. In prokaryotic organisms such a growth pattern is often associated with nutritional depletion of the medium (Schaechter, Maaloe & Kjeldgaard, 1958). This finding, taken in conjunction with the observation that cells which demonstrate contact inhibition of growth show a progressive and striking decrease in the rate of protein and RNA synthesis (Levine, Becker, Boone & Eagle, 1965), suggested that a situation similar to 'stringent' control (Edlin & Broda, 1968) of RNA synthesis in bacteria may apply to cells of higher organisms as well. As in bacteria, amino acid deprivation may play a pivotal role. In recent years it is apparent that other laboratories have also noted the resemblance of the phenomenon noted in prokaryotes (Maden, Vaughan, Warner & Darnell, 1969; Ellem, Fabrizio & Jackson, 1970; Smulson & Thomas, 1969; Shields & Korner, 1970; Bölesföldi, Poels & Eliasson, 1971; Tiollais, Galibert & Boiron, 1971; Hershko, Mamont, Shields & Tomkins, 1971).

If this type of control pertains to prokaryotic cells and in some manner plays a role in contact inhibition, we may be able to demonstrate that the transformed 3T3T, which is not contact-inhibited, is less affected by varying degrees of amino acid deprivation. Furthermore, as was previously seen (Meisler, 1973), unbalanced growth manifests itself very early after exponential cell increase has begun and is more
pronounced in 3T3. A difference in growth rate may be demonstrable therefore, even in complete medium, if the medium is not changed daily.

Accordingly, in the experiments to be described, the growth rate constants were computed for the cells grown at low population density in Dulbecco's modified Eagle's Medium in which a single amino acid was decreased 1000-fold in concentration. Since it was felt that calf serum might contain a source of amino acids which had been non-specifically adsorbed, the calf serum was passed through Sephadex columns as described below before being used.

Our results indicate that neither cell was able to grow appreciably if the concentration of glutamine, cystine or arginine was reduced by a factor of 1000. For the remaining amino acids, with the single possible exception of tryptophan, the 3T3T showed a decidedly superior initial growth rate. This could also be demonstrated in complete medium with normal 10% calf serum, but not if the medium was changed daily or the serum previously treated with Sephadex.

MATERIALS AND METHODS

Cell culture

These methods have been outlined in detail (Meisler, 1973). Cells were plated in 60-mm plastic Petri dishes (Falcon) in complete Dulbecco's modified Eagle's Medium (Grand Island Biological Co.) containing 10% calf serum (Colorado Serum Co.) at approximately \(2 \times 10^6\) cells per plate. They were grown in a humidified incubator at 37 °C in an atmosphere of 10% CO₂. About 8 h later the medium was changed, again with complete medium. The next day, the medium was again changed, this time containing all the components of complete medium with the following alterations. All plates contained 4 ml of medium; the serum had previously been passed through a 30 x 1 cm column of Sephadex G 25 at 4°C, from which the void volume was collected; a single amino acid was decreased in concentration by a factor of 1000. Paired plates were also followed as controls, in which the full complement of amino acids was present. Cell growth was monitored until it was determined that exponential increase was no longer occurring.

Determinations of the growth rate constant

Duplicate plates were counted for each time point and the average plotted on semilogarithmic graph paper. The exponential growth rate constant, \(k\), was calculated from the standard first-order equation for growth:

\[
\frac{dN}{dt} = kN.
\]

Solving by integration between limits, on rearranging terms, the following equation used in our calculations is obtained

\[
k = \frac{2.3 \log \frac{N}{N_0}}{T},
\]

where \(N =\) cell number and \(T =\) time in hours.

The experiments were repeated to ensure that \(\Delta N\) was being measured over a period in which the cell increase was exponential. Growth was measured at 8-16 h intervals, until it was clear that at least 3 points fell on a straight line in a semilog plot. Generally, 2 growth curves were required to be certain that exponential growth had been achieved. From experiment to experiment, there were slight differences in the absolute values of the growth rate constants presumably due to variation in the quality of the cells, age of the medium and temperature fluctuations. The differences were of the order of ± 10%.
Growth of \(3T_3\) during amino acid deprivation

Counting of cells

Duplicate plates were aspirated and washed twice in Tris A (NaCl, 138 mM; KCl, 50 mM; Na\(_2\)HPO\(_4\), 0.7 mM; tris(hydroxymethyl)aminomethane, 25 mM pH 7.4). They were trypsinized using 0.1% trypsin (Difco) and suspended in an adequate volume for counting in Ca\(^{2+}\) - and Mg\(^{2+}\)-free Earle's Balanced Salt Solution. The counting was performed in a Cellscope Particle Counter (Particle Data, Inc., Elmhurst, Ill.) equipped with a 100-μm orifice. All counts were performed in duplicate.

Preparation of labelled protein for determination of proteolytic activity

*E. coli* B was grown to a density of 5 x 10^8 cells/ml in a volume of 1 l. The flask was inoculated with a 100 μCi of a ^14^C-amino acid mixture and allowed to grow for an additional 4 h. The cells were spun down and resuspended in about 120 ml of buffer and sonicated. The cell debris and ribosomes were removed by centrifugation and the supernatant treated with an excess of ribonuclease and deoxyribonuclease. The supernatant was dialysed for 24 h in the cold against buffer containing 10 mM tris(hydroxymethyl)aminomethane and 50 mM KCl, pH 7.0. The dialysate was then passed through a 30 x 10 cm column of Sephadex G-15 and the void volume collected. The final protein product contained 400 dpm/μg protein which in the presence of carrier albumin was 98% precipitable by 5% trichloroacetic acid containing 0.25% sodium tungstate.

Amino acid transport

Cells were inoculated into 100-mm Petri dishes containing previously washed and heat-sterilized 15-mm circular glass coverslips, no. 2 thickness (Corning). Sufficient cells were seeded to ensure exponential growth at the start of the experiment. The medium was changed 12 h later and on alternate days until used. All cells were fed 24 h before the start of the experiment. Prior to the transport measurements sufficient coverslips were set aside to provide 6 separate determinations of cell number (Meisler, 1973). The experiment was not performed unless the standard deviation of the mean count was 10% or less.

At the start of the experiment the growth medium was aspirated and the coverslips washed twice in Tris A. They were then placed in a Petri dish with 4 ml of Tris A containing 0.1% glucose, all the amino acids present in growth medium at their usual concentration and 0.2 μCi/ml of the radioactive amino acids to be tested. Initial rates of uptake were determined by removing coverslips at 15-s intervals for 1 min. At least 10 coverslips were removed at each time point for the determination of radioactivity.

After being removed from the incubation medium, the coverslips were submerged twice in 2 successive beakers containing Tris A, drained by touching the edge to a piece of filter paper, and added to a scintillation vial. The cells were dissolved in 0.5 ml of NCS (Amersham/Searle) and 10 ml of toluene-based scintillation fluid added. The composition of the scintillant as well as the counting procedure have been described previously (Meisler, 1973).

Non-specific adsorption of radioactivity to cells was corrected for by immersing duplicate coverslips in the incubation media, and removing them in about 1 s, processing as described above, and subtracting their retained radioactivity from the value at each time point. Frequently the amounts of radioactivity retained by these 'zero' controls were obviously too high; in these cases the lines were extrapolated to zero time and the slope determined. The rate of transport was expressed as a function of the cell water. The latter was determined as already described (Meisler, 1973). Radioactivity in dpm was converted to nmol from the known specific activity of the amino acid in the medium.

Measurement of radioactivity

Liquid scintillation counting was performed as previously described (Meisler, 1973). Liquid samples were counted in the toluene-based scintillant with appropriate quantities of NCS (Amersham/Searle) added.
RESULTS

Effect on cell growth characteristics of deficient medium

In the following experiments about $2 \times 10^5$ cells were plated on to 60-mm Petri dishes in normal medium. The zero time point coincided with the time at which experimental medium was added. The curves shown are those in which phenylalanine was varied. The differences obtained by varying other amino acids are not shown for the sake of brevity but will be commented upon.

Fig. 1 demonstrates the effect of Sephadexed serum on the growth of the cells. In complete medium containing normal serum, $3T_3T$ show a distinctly higher initial growth rate than $3T_3$. This difference is abolished using Sephadexed serum. The effect is due almost completely to a decrease in the rate of growth of the $3T_3T$ with almost no change in the $3T_3$. On the other hand, both types of cells show decreased cell yield which is more marked for $3T_3T$ than $3T_3$. It can be seen, to some extent in Fig. 1 and more markedly in Fig. 2, that once the maximum cell number is reached, some of the cells begin to die. This is a variable finding and not present in all experiments. It will not be discussed here since our concern is with initial growth rates. It should at least be noted, however, that after 48 h of growth, the medium from $3T_3T$ cultures is much more acidic than that from $3T_3$. Therefore, in referring to cell yield, we shall be referring to the maximum number of cells attained prior to the decline.

In Fig. 2, the cells were grown in medium containing either Sephadexed or normal serum and the concentration of phenylalanine was reduced by a factor of 1000. In
Growth of 3T3 during amino acid deprivation

Fig. 2. The effect of partial phenylalanine deprivation (1000-fold reduction in concentration to 0.4 μM) on growth of 3T3 and 3T3T in medium containing normal or Sephadexed calf serum. •—• 3T3, ■—■ 3T3T, normal serum; O—O 3T3, □—□ 3T3T, Sephadexed calf serum. The graph is semilogarithmic.

Table 1. Comparisons of growth rate constants of 3T3 and 3T3T grown in medium containing Sephadexed serum and a single amino acid reduced 1000-fold in concentration

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>α_{3T3}</th>
<th>α_{3T3T}</th>
<th>\frac{α_{3T3}}{α_{3T3T}}</th>
<th>α_{3T3}</th>
<th>α_{3T3T}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine (0.40)</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cystine (0.80)</td>
<td>0.0073</td>
<td>0</td>
<td>—</td>
<td>0.27</td>
<td>0</td>
</tr>
<tr>
<td>Glutamine (0.40)</td>
<td>0.0062</td>
<td>0</td>
<td>—</td>
<td>0.23</td>
<td>0</td>
</tr>
<tr>
<td>Phenylalanine (0.40)</td>
<td>0.020</td>
<td>0.036</td>
<td>0.55</td>
<td>0.63</td>
<td>0.90</td>
</tr>
<tr>
<td>Tyrosine (0.40)</td>
<td>0.018</td>
<td>0.034</td>
<td>0.57</td>
<td>0.64</td>
<td>0.99</td>
</tr>
<tr>
<td>Leucine (0.80)</td>
<td>0.019</td>
<td>0.034</td>
<td>0.57</td>
<td>0.62</td>
<td>0.86</td>
</tr>
<tr>
<td>Glycine (0.40)</td>
<td>0.022</td>
<td>0.039</td>
<td>0.69</td>
<td>0.90</td>
<td>1.05</td>
</tr>
<tr>
<td>Serine (0.40)</td>
<td>0.022</td>
<td>0.032</td>
<td>0.69</td>
<td>0.76</td>
<td>0.88</td>
</tr>
<tr>
<td>Lysine (0.80)</td>
<td>0.022</td>
<td>0.030</td>
<td>0.73</td>
<td>0.60</td>
<td>0.75</td>
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<tr>
<td>Threonine (0.80)</td>
<td>0.030</td>
<td>0.037</td>
<td>0.80</td>
<td>0.85</td>
<td>1.01</td>
</tr>
<tr>
<td>Valine (0.80)</td>
<td>0.021</td>
<td>0.023</td>
<td>0.89</td>
<td>0.78</td>
<td>0.88</td>
</tr>
<tr>
<td>Isoleucine (0.80)</td>
<td>0.028</td>
<td>0.031</td>
<td>0.89</td>
<td>0.79</td>
<td>0.84</td>
</tr>
<tr>
<td>Histidine (0.20)</td>
<td>0.026</td>
<td>0.031</td>
<td>0.84</td>
<td>0.74</td>
<td>0.83</td>
</tr>
<tr>
<td>Methionine (0.20)</td>
<td>0.030</td>
<td>0.029</td>
<td>1.02</td>
<td>0.87</td>
<td>0.90</td>
</tr>
<tr>
<td>Tryptophan (0.08)</td>
<td>0.038</td>
<td>0.031</td>
<td>1.21</td>
<td>1.13</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* Growth rate constant in h⁻¹.
† α is the growth rate constant in limiting amino acids. α₀ is the constant in complete media.
All media contained the identical lot of Sephadexed serum.
‡ Concentration of the amino acid in complete medium (mM).
Fig. 3. The effect of serum on the transport of phenylalanine in 3T3 (A) and 3T3T (B). The population density of 3T3 was $2.5 \times 10^4$ and of 3T3T, $1.8 \times 10^4$ cells/cm$^2$. Vertical bars indicate s.d. ●—●, control, no serum; ○—○, Sephadexed, fresh serum; ■—■, normal, fresh serum. The slopes of the curves, representing rates of transport in nmol/μl cell H$_2$O/min are: A, ●, 175; ○, 180; ■, 190; and B, ●, 150; ○, 183; ■, 295.

These cultures, the initial growth rates using normal serum are essentially the same as in medium containing the full complement of amino acids. Only the maximum yield of cells is depressed and the depression for the 3T3T is almost twice that for the 3T3. In medium containing Sephadexed serum and reduced phenylalanine, the initial growth rate of 3T3T is unchanged from that obtained with Sephadexed serum alone, but the growth rate of 3T3 is now decreased by 40%. As will be shown, in medium prepared with Sephadexed serum, partial deprivation of any amino acid, with the possible exception of tryptophan, decreases the initial growth rate of 3T3. The effect on the growth rate of 3T3T is variable and depends on the specific amino
Growth of 3T3 during amino acid deprivation

Fig. 4. The effect of incubation at 37 °C on the ability of serum to stimulate the transport of phenylalanine. The population density of 3T3T (A) was $1.4 \times 10^4$ and of 3T3T (B), $3.5 \times 10^4$ cells/cm². Vertical bars indicate S.D. •—•, control, no serum; O—O, fresh, normal serum; ■—■, normal serum incubated at 37 °C for 24 h prior to the experiment; □—□, normal serum incubated for 72 h at 37 °C prior to the experiment. The slopes of the curves representing rates of transport in nmol/ml cell H₂O/min are: A, •, 4.3; O, 5.1; □, 4.4; and B, •, 5.7; O, 5.5; ■, 9.1; □, 3.7.

acid (Table 1). The maximum yield of both cell types is further depressed, below that seen with complete medium and Sephadexed serum. The effect of this depression varied with the amino acid but is more pronounced for the 3T3T.

Finally, one further experiment not shown here should be noted. If the cells are completely deprived of phenylalanine and grown with normal serum, the initial growth rates of both types become identical as in complete medium with Sephadexed serum (Fig. 1). However, the rate is now profoundly depressed, much like the 3T3.
grown in medium containing Sephadexed serum with 1000-fold less phenylalanine (Fig. 2). There is also sharp reduction in cell yield and evidence of cell death as judged by a decline in cell number after a maximum has been reached. It is of interest, though, that growth continues for 25–30 h.

Effect of serum on the transport of phenylalanine

The cells were in exponential growth at the time of the experiments and the population densities are recorded in the legends to the figures. The incubation medium was adjusted to the proper serum content just prior to the addition of the cell-laden coverslips. All serum samples were obtained from the identical bottle of calf serum. Under these experimental conditions, accumulation of phenylalanine is completely inhibited when the incubation medium is made 10 mM in iodoacetate. Fig. 3 compares the rate of transport of phenylalanine in incubation media containing no serum, Sephadexed serum and normal serum for 3T3 and 3T3T. Entirely similar results have been obtained with lysine. It is apparent that serum addition has only a small stimulating effect on the rate of transport of the amino acid for 3T3 while there is a marked stimulation in the case of 3T3T. Passing the serum through a Sephadex column prior to use essentially abolished the stimulation noted for 3T3T. The slight stimulation with Sephadexed serum noted in Fig. 3B is variable and often entirely absent.

We have also tested the effect of ageing on the ability of serum to stimulate amino uptake in 3T3T. The results using phenylalanine are shown in Fig. 4. In these experiments, aliquots of serum were removed from the stock bottle and placed at 37 °C for the indicated time. In Fig. 4A, a small stimulatory effect of fresh serum is noted for 3T3; this is a constant finding but does not usually exceed 15%. The 20% stimulation in Fig. 4A is the highest we have thus far seen. Serum which has been aged for 3 days has no effect at all. In contrast, Fig. 4B demonstrates that fresh serum markedly stimulates uptake of the amino acid in the case of 3T3T. A somewhat lesser effect is noted for serum which has been aged for 24 h, and the effect is completely abolished when the serum is aged for 72 h. Further experiments have indicated that serum frozen for 9 months or longer has lost the ability to stimulate amino acid uptake. We have not tested the stimulating capacity of serum which has been stored in the frozen state for less than this period of time. Our data are also insufficient to comment on the effect of aged, frozen serum on the cell yield.

Proteolytic activity

In order to perform the amino acid deprivation experiments described, all possible sources of exogenous amino acids must be controlled. If, for example, one cell type could partially degrade the protein in the medium by secreting a proteolytic enzyme, an apparent growth advantage could be demonstrated. That such was not the case was demonstrated in the following experiments.

A series of plates were inoculated with 10⁶ cells and at the appropriate time the medium was changed and 3 ml of either complete medium or medium with reduced
Growth of $3T_3$ during amino acid deprivation

Lysine was added. Both media contained Sephadexed serum. At 24-h intervals the medium was removed and 0.5 ml added to a tube which contained 20 cold amino acids and 12 680 dpm of $^{14}$C-labelled $E. coli$ protein prepared as already described. The total volume was 0.6 ml and incubation was carried out at 37 °C for 4 h. At the end of the incubation time the reaction mixture was precipitated with 0.25 ml of 10% trichloroacetic acid containing 0.5% sodium tungstate. Control tubes were immediately precipitated on the addition of medium, without incubation. Another control contained fresh medium incubated as in the experimental tubes. A final control contained 0.0005% trypsin in place of medium. The supernatants of the reaction mixture were obtained by centrifugation and 100-μl samples counted in duplicate in the scintillation counter.

While the tubes containing trypsin completely solubilized the radioactive bacterial protein, no solubilization at all was detected with medium obtained from plates which contained the cells. The cultures in complete medium were followed for 120 h and attained cell numbers greater than $10^6$ per plate. The cultures in deficient medium could only be followed for 96 h and only the transformed cells exceeded $10^6$ per plate.

In other experiments it was shown that $3T_3$ or $3T_3T$ cells grown on coverslips in the same Petri dish as cells whose protein had been previously labelled with a radioactive amino acid, contained no acid-insoluble radioactivity after 80 h of incubation. Finally, cells grown for 80 h in medium to which radioactive $E. coli$ protein had been added also showed no uptake of label.

Growth rates in the presence of amino acid deprivation

Table 1 (p. 865) shows the growth rate constants for $3T_3$ and $3T_3T$ grown in medium in which the concentration of the particular amino acid is reduced by a factor of 1000. As was shown in Fig. 1, the initial growth rates are almost identical when $3T_3$ and $3T_3T$ are grown in medium containing Sephadexed serum. The Roman numerals at the left of the table indicate a somewhat arbitrary classification which will be elaborated upon in the Discussion. For some amino acids as many as 5 individual growth curves were determined, but in others, where the accuracy appeared to warrant it, only 2 were made. The constants obtained are the average of two to three determinations.

DISCUSSION

The experiments reported here and in the preceding paper (Meisler, 1973) provide a rationale for the detailed investigations we have made on the effect of amino acid lack on cell growth. Succeeding papers will be devoted to demonstrating the effect of intracellular amino acid depletion on the synthesis of ribosomal RNA and protein. We believe that the data in Table 1 substantiate our belief that for most amino acids present in this medium, partial deprivation has a greater adverse effect on the growth rate of $3T_3$ than of $3T_3T$.

It can be seen in Figs. 1 and 2 that the effect of amino acid deprivation on growth
rate is not evident unless Sephadexed serum is used. Recall, however, we indicated that complete deprivation of phenylalanine causes both cell types to grow as did the 3T3T in medium in which the serum is Sephadexed and phenylalanine lowered 1000-fold. We therefore postulated that a major effect on growth rate of passing the serum through Sephadex G 25 was due to removal of a factor which stimulates amino acid uptake in 3T3T and to a much lesser extent in 3T3. It appears that the reason the initial growth rate is unaffected in medium containing normal serum with 1000-fold less phenylalanine is that the deprivation was not sufficiently stringent. However, in medium in which this hypothetical serum factor is removed, the differential effect of amino acid deprivation becomes evident.

Fig. 3 lends support to the existence of this factor and demonstrates its removal by passage through a Sephadex column. In Fig. 4 we have demonstrated that its effectiveness is completely abolished after a 3-day incubation at 37 °C.

In recent years the subject of serum growth factors has come under close scrutiny by many investigators. The topic has been reviewed in a recent symposium (Wolstenholme & Knight, 1971). A wide array of putative factors have been partially characterized which differentially affect cell yield and viability of either contact-inhibited cells or their transformed counterparts. The assay for these factors tends to lack precision because cell yield and viability must depend upon a multiplicity of factors. While more than a single factor must determine growth rate, or better still, the rate of amino acid uptake, nevertheless in cells which are growing exponentially, in relatively fresh medium, within 36 h of plating, it might be easier to distinguish primary events more clearly. The factor(s) described in this report may very well be identical to one already shown to increase the cell yield of 3T3T (Paul, Lipton & Klinger, 1971).

A further effect that remains to be explained is why the growth rate of the 3T3T is reduced in Sephadexed serum but that of 3T3 is not. It is evident from Fig. 3 that in the absence of serum the rate of transport of phenylalanine is far greater in 3T3T than in 3T3. Similar findings have been reported by several investigators for α-aminoisobutyric acid and cycloleucine under somewhat different conditions (Isselbacher, 1972; Foster & Pardee, 1969). Hare (1967) found no definite changes in the characteristics of phenylalanine transport by virus-transformed hamster cells. This may have been due to inadequate corrections for changes in cell volume. We have data on the transport of 6 different amino acids which demonstrate that at low population densities (less than $4 \times 10^4$ cells/cm$^2$), during exponential growth, the rates of transport in 3T3T are significantly higher than in 3T3 (manuscript in preparation). It appears that a maximal growth rate of 3T3T can be achieved only with extremely high transport rates. Sephadexed serum is devoid of the factor which permits such a rate to be reached.

Each of the constants shown in Table 1 is the average of just a few values (two or three) which differ at most by about 10%. In view of the variability in the condition of the cells and the differences in the age of the medium from experiment to experiment, it was surprising how reproducible the curves were. The condition of the cells and medium was judged by comparing growth in complete medium to the best value
Growth of \(3T_3\) during amino acid deprivation

obtained in all the experiments in which \(\alpha\) (growth rate constant in complete medium) differed by more than 15%.

The basis for grouping the amino acids is the value obtained in column 4, \(\alpha3T_3/\alpha3T_3\).

Group I: Essentially no growth of either cell type with partial deprivation.

Group II: Growth rate of \(3T_3\) 40-50% greater.

Group III: Growth rate of \(3T_3\) 25-35% greater.

Group IV: Growth rate of \(3T_3\) 10-20% greater.

Group V: No difference (methionine) or growth of \(3T_3\) greater.

At present, the metabolic basis for this distribution of amino acids is not clear. The question of why, for example, \(3T_3\) grows so much more poorly in the presence of reduced phenylalanine than of isoleucine (Table 1) is obscure. On the other hand, we shall present evidence in a succeeding paper to show that the transformed cell is able to extract most of these amino acids from the medium with greater efficiency than the \(3T_3\).

For the sake of completeness, there is another important factor that may influence these studies on amino acid deprivation. It is conceivable that a cell may derive a growth advantage in medium deficient in amino acid, if it were able to utilize the amino acids contained in its own protein. In some way it would be able to regulate its rate of protein turnover in response to amino acid need. Pulse-chase type experiments in which estimates of the average protein half-life are made by labelling cells with amino acids and then following the diminution of precipitable radioactivity, or alternatively by adjusting conditions so that there is no net increase in cell protein and determining 'excess' amino acid incorporation, have been performed. For both \(3T_3\) and \(3T_3\) an average turnover rate of 0.5-1.0% per h was found, similar to that found by other workers (Eagle, Piez, Fleishman & Oyama, 1959) using different cell lines. These methods, however, are not reliable because of recycling of the internal amino acids (Klevecz, 1971; Righetti, Little & Wolf, 1971). Preliminary work in our laboratory, using more reliable methods, has suggested that \(3T_3\) has a higher average rate of turnover than \(3T_3\). The role of this increased turnover in the economy of the cell is not clear at present.

Finally, we should compare our results with those obtained in the detailed studies of Griffiths (1970, 1971). This investigator employed the diploid human cell line WI-38 while we have used a heteroploid mouse line. Our results have been obtained using medium containing Sephadexed serum; however, even fresh serum does not appear to stimulate the growth of WI-38 to the same extent as it does \(3T_3\) (Holley & Kiernan, 1968). The WI-38 appear to be able to attain population densities of \(1 \times 10^8\) cells/cm² with medium change every 3-4 days while with \(3T_3\) this can only be attained by special manipulations (Meisler, 1973). A final difference is that Griffiths was measuring cell yield while we measured initial growth rate.

Nevertheless, while differences do exist in our findings, there are striking similarities. For this purpose column 5 in Table 1 should be consulted. For both \(3T_3\) and WI-38 cells, arginine, cystine and glutamine are highly essential. Griffiths also includes in this category leucine and isoleucine. Our next category includes phenyl-
alanine, tyrosine, leucine and lysine while for WI-38 it is lysine, histidine and valine. For the transformed cells, 3T3T (column 6, Table 1), the first category is the same as for 3T3 whereas lysine, valine, isoleucine and histidine fall into the second category.

We concur with Griffiths' formulation that amino acids may play a vital role in contact inhibition, despite the fact that the medium is in no way depleted of these when growth ceases. The mechanism of this growth inhibition may have to do with changes in the plasma membrane that occur with increasing population density which prevents the uptake of essential small nutrients by the 3T3 cell. Though we can duplicate many of the changes found in contact-inhibited cells, by partial amino acid deprivation, this work in no way establishes a cause-and-effect relationship. In the previous paper (Meisler, 1973), we suggested that nutritional deficiency may play a role in this phenomenon. In this paper we have indicated that an exogenously imposed deficiency of amino acids can duplicate the growth patterns seen during contact inhibition.

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REFERENCES


Hare, J. D. (1967). Location and charactenstics of the phenylalanine transport mechanism in normal and polyoma-transformed Hamster cells. Cancer Res. 27, 2357-2363.


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