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Control of the Uptake of Amino Acids by Serum in Chick Embryo Cells, Untransformed or Transformed with Rous Sarcoma Virus

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Summary. Forty to fifty minutes after removal of serum, the net total uptake of amino acids in growing secondary cultures of normal or virus-transformed chick embryo cells, stopped or proceeded only at a highly reduced rate. In both normal and transformed cells, the *initial* (0–40 min) rate of the above uptake was the same in the absence of serum as in its presence. The initial rate of the total uptake of amino acids in growing transformed cells was about the same as in growing normal cells. Neither in the normal nor in the transformed cells was the rate of the total uptake of amino acids reduced by cell confluence alone. In highly dense, hyperconfluent cultures of normal cells in which cell growth was arrested, the rate of uptake in the absence or in the presence of serum was four- to fivefold lower than the rate obtained in growing normal cells under similar conditions; in the absence of serum, the net uptake stopped after 40 min in the hyperconfluent cultures as well. It appears that cells growing in tissue culture require a serum factor for maintenance of the required high rates of uptake of amino acids and that the inhibition of growth at high cell densities is a result of depletion of this factor from serum, or the inability of the cells in a dense culture to respond to the factor. A serum factor is apparently also required for maintenance of the reduced rates of uptake of amino acids observed in hyperconfluent cultures.

Available evidence, e.g. for regenerating liver [8], lymphocytes [2, 17, 25, 31, 32, 36] and BHK cells [3], suggests that when resting mammalian cells are stimulated to divide, one of the earliest events to occur is a several-fold increase in the rates of transport of nutrients. Further, the rates of transport of nutrients in dividing mammalian cells appear to be about an order of magnitude greater than in homologous resting cells (e.g. ref. [5]). In this paper we have studied the effect of serum on the rate of the net total uptake in chick embryo fibroblasts of several amino acids which are not made by the cell and do not represent common metabolic pools; we show that serum is essential for the maintenance

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of these rates at the high level necessary for growth of these cells in tissue culture. Although an overall stimulatory effect of serum on transport is well-established [11–14, 21, 30], in only a few earlier studies was the effect of serum on the transport of nutrients studied under conditions required for sustenance of cell growth. Griffiths [18] demonstrated the stimulatory effect of serum on the uptake of amino acids in limiting cultures, and Adamson, Herington and Bornstein [1] showed that serum stimulates the intracellular accumulation of amino acids by stimulating the formation of an amino acid-membrane complex. These observations are in agreement with the general conclusions arrived at in the present study.

We also show here that the rates of the net total uptake of amino acids decrease by a factor of 4–5 when normal chick embryo cells become hyperconfluent and stop growing. Our observations suggest that serum may exercise its stimulatory effect on growth in tissue culture, at least partly through interference with a mechanism operative in resting mammalian cells which restrains the uptake of essential nutrients.

Materials and Methods

Cell Culture

The normal cells used were secondary cultures of chick embryo fibroblasts (CE cells), obtained by subculturing 4- to 6-day-old primary cultures of single, whole, lymphomatosis-free, Brown Leghorn chick embryos [15]. The primary cultures were grown in 10-cm Falcon dishes in Eagle's minimum essential medium containing double the normal concentration of amino acids and vitamins, 10% tryptose phosphate broth (TPB, Difco), 5% calf serum, and antibiotics (penicillin, streptomycin, kanamycin). Subculturing of 1×10^6 primary CE cells in 6-cm Falcon dishes yielded low density, nonconfluent or subconfluent, actively growing cultures ($1–3 \times 10^6$ cells/dish) 1–3 days later; subculturing of $3–4 \times 10^6$ CE cells on the 6-cm dishes yielded high density, confluent, slow-growing or nongrowing cultures ($6–10 \times 10^6$ cells/dish) 3 days later. It has been shown earlier that under the experimental conditions used here, CE cells become confluent at about 4×10^6 cells/dish in 6-cm dishes [16]. Cells continued to grow in confluent cultures containing $6–7 \times 10^6$ CE cells, though at a slower rate than in nonconfluent cultures; the growth was totally arrested in hyperconfluent cultures containing 10^7 cells.

The virus-infected (transformed) cells used were also secondary cultures of CE cells, derived from primary cells infected, at the time of plating, with a cloned, high-titer isolate of Schmidt-Ruppin strain Rous sarcoma virus (SR-RSV-D) [6]. Over 50% of the cells were transformed and producing virus at the time of subculturing, and over 80% at the time of the experiments which were carried out in parallel on replicate infected and uninfected cultures. The medium used for the infected cultures was the same as for the uninfected cultures.

All cultures were grown at 37 °C in airtight boxes containing a water-saturated atmosphere of 95% air plus 5% CO_2.

Labeled Amino Acids

High specific activity, tritium-labeled leucine, lysine, valine and arginine were obtained from the Commissariat a l'Energie Atomique (Saclay, France). The concentration of the unlabeled amino acids present in the growth medium was not significantly altered by the amount of the labeled amino acid added for the uptake studies.

Measurement of Uptake

The cells, grown to the desired density, were washed twice, each time with 5 ml of serum-free growth medium warmed to 37°C. Addition or removal of the medium was done with a pipette, and the original medium in which the cells were grown was saved and stored at 37°C. After the two washings, 2.5 ml of the appropriate medium (the serum-free growth medium used for washing or the original medium which had been stored) was added, and followed immediately by the labeled amino acid in 0.05–0.10 ml of 0.9% NaCl. The dishes were then incubated as above at 37°C in the airtight boxes used for growing the cells. At the end of the incubation period (up to which time all the operations were carried out in a 37°C room), the cultures were chilled and washed rapidly 5 times, by decantation, each time with 5 ml of cold Dulbecco's phosphate-buffered saline containing azide and the unlabeled amino acid [3]; the entire washing took less than 3 min. It was shown in several experiments that the last wash contained less than 1% of the radioactivity contained in the cells after this wash. The pattern of fall of radioactivity in the serial washes did not indicate any significant leaking out of the labeled amino acids taken up by the cells.

The cultures were then incubated with 1.5 ml of a trypsin-containing solution [3] for 5–10 min at 37°C, until all the cells were detached as revealed by microscopic examination. The cell suspension was transferred to 5-ml centrifuge tubes and the dishes rinsed with 1 ml of azide-PBS. Two ml of 12% trichloroacetic acid (TCA) was added, the mixture allowed to stand in the cold for a few hours and then centrifuged. Radioactivity was determined in 0.5 ml of the supernatant to arrive at the total radioactivity in the acid-soluble fraction. The residue was washed twice with 5% TCA by centrifugation and dissolved in 2 ml of 0.6 N NaOH. Radioactivity was determined in 0.2 ml of the solution to arrive at the total incorporation in the acid-insoluble fraction. Radioactivity measurements were carried out as described earlier [3].

It was shown that CE cells incubated at 37°C in the serum-free growth medium for 180 min (the maximum period of incubation used here), retained their viability as judged by the exclusion of trypan blue.

The uptake values are given on the basis of the protein content of the dishes, as protein estimations gave a more reliable estimate of the cell density than did cell counting. Protein was estimated by Folin's reagent in 0.1–0.5 ml of the alkali solution. The sum of the radioactivity in the acid-soluble and the acid-insoluble fractions was taken to represent the total uptake of the amino acid; unless otherwise mentioned, the term "uptake" refers to the total uptake. As the back flow was not estimated, the uptake values refer to the net uptake.

Results

Effect of Serum on the Uptake of Amino Acids by Growing Normal and Virus-Transformed Cells

Figs. 1 and 2 show the net total uptake of four ^{3}H-labeled amino acids (arginine, leucine, lysine and valine) for periods up to 70 min in

the absence of serum and the distribution of the radioactivity taken up in the acid-soluble and the acid-insoluble fractions, in normal and RSV-transformed CE cells grown to two different cell densities, one below cell confluence and the other above confluence but still permitting cell growth. Up to 40 min, the total uptake increased with time in all the cases, linearly in some and nonlinearly in the others. At 40–50 min, the uptake stopped or its rate was drastically reduced, at both cell densities in normal cells and at the lower cell density in transformed cells. In transformed cells at the high cell density, the uptake of all the amino acids continued beyond 40 min but at reduced rates. No significant or consistent differences were found between growing normal and transformed cells in regard to the rates of the total uptake of amino acids.

The virtual cessation of or reduction in the net total uptake of amino acids at 40–50 min in the absence of serum was not due to cessation of protein synthesis, as synthesis of protein from the amino acids taken up continued all through the duration of the experiment in normal as well as in transformed cells (Figs. 1 and 2; *see also* Table 1); consequently, the total radioactivity in the acid-soluble pool fell after 20–40 min, the extent of drop from the maximum value attained at 20 or 40 min being the least for transformed cells at high cell density. As labeled amino acids were used at concentrations at which they were normally present in the tissue culture medium for growth of the cells, the above cessation of uptake could not be due to depletion of the precursor. In the presence of serum, the uptake of amino acids in growing cultures of normal CE cells at both low and high cell densities continued almost linearly

Fig. 1. The time-course of uptake of valine and leucine by normal CE cells and by RSV-transformed CE cells, at low and high cell densities. The cells were washed on the petri-dish with serum-free growth medium, incubated for the specified time in the serum-free medium with the ^{3}H-amino acid (22.0×10^6 cpm), washed again, released from the dish by trypsin, and processed for the estimation of uptake (total uptake and that into the acid-soluble and the acid-insoluble fractions taken separately) as described in the text. A separate petri dish was used for each time point, each amino acid and each cell density. The amount of protein per petri dish varied from 214–374 (mean 302 ± 13) μg for normal cells at low cell density (○), 585–891 (mean 688 ± 21) μg for normal cells at high cell density (●), 221–381 (mean 310 ± 11) μg for transformed cells at low cell density (△), and 641–780 (mean 706 ± 10) μg for transformed cells at high cell density (▲). Low cell density: $\sim 3 \times 10^6$ cells; high cell density: $6–7 \times 10^6$ cells. (*A*) Total uptake (acid-soluble + acid-insoluble); (*B*) radioactivity in the acid-soluble fraction; (*C*) radioactivity in the acid-insoluble fraction. The data given are of a typical experiment

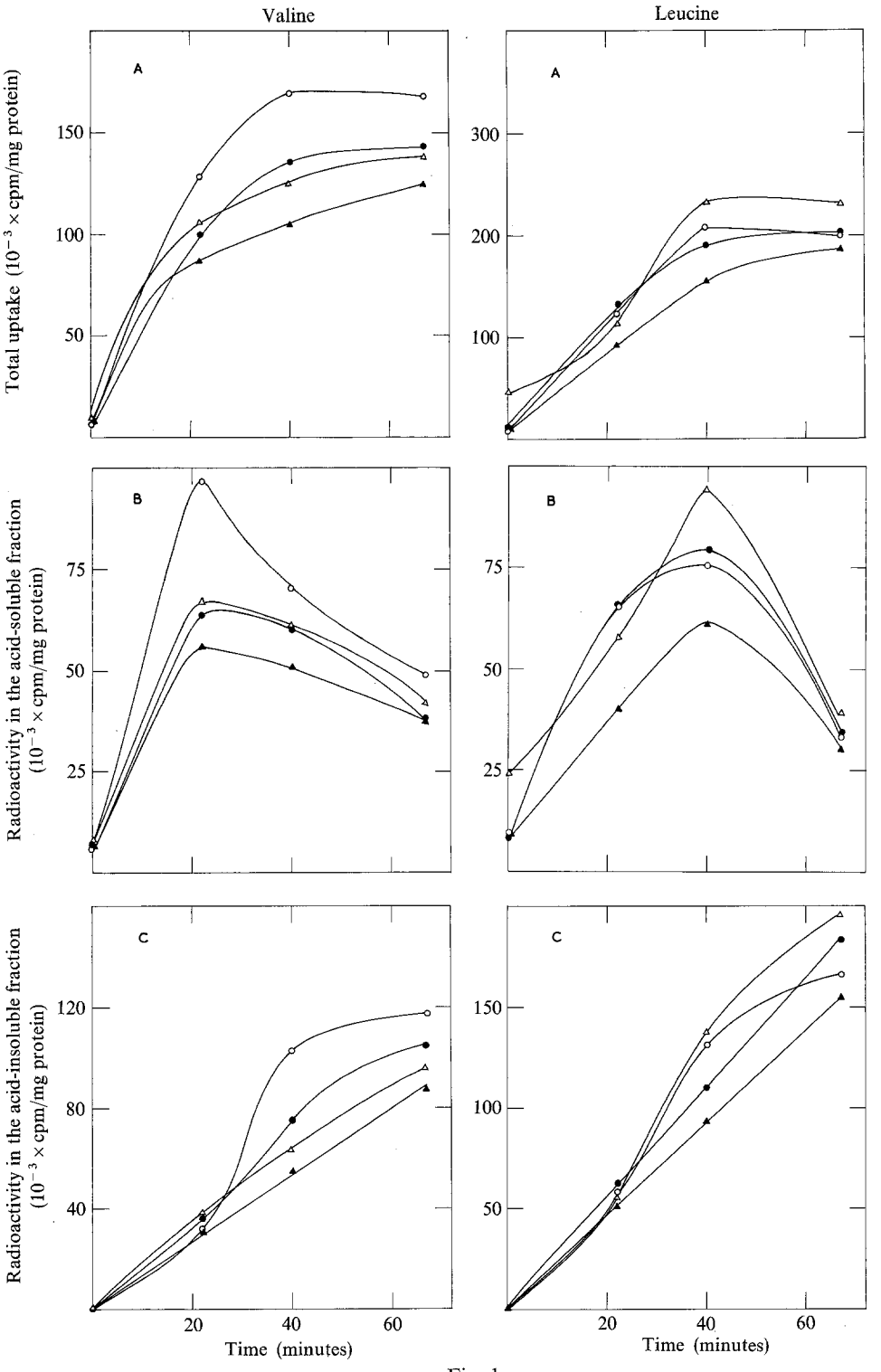
Valine
Leucine
A
B
C
Total uptake (10^{-3} × cpm/mg protein)
Radioactivity in the acid-soluble fraction (10^{-3} × cpm/mg protein)
Radioactivity in the acid-insoluble fraction (10^{-3} × cpm/mg protein)
150
100
50
300
200
100
75
50
25
120
80
40
20
40
60
Time (minutes)

Fig. 1

Table 1. Comparison of the uptake of valine and arginine by nonconfluent growing cells and by hyperconfluent nongrowing cells, in the presence and in the absence of serum[a]

^{3}H-amino acid	Nature of the culture	Medium of incubation	Time of incubation (min)	Uptake (10^{-3} × cpm/mg protein)		
				Acid-soluble	Acid-insoluble	Total (acid-soluble + acid-insoluble)
Arginine	Growing	+Serum	42	58.3 (50.2)	57.8 (49.8)	116.1
			82	73.7 (32.2)	155.0 (67.8)	228.7
		−Serum	42	54.5 (47.6)	60.0 (52.4)	114.5
			82	25.3 (22.0)	89.4 (78.0)	114.7
	Nongrowing (hyperconfluent)	+Serum	42	8.5 (39.2)	13.2 (60.8)	21.7
			82	18.3 (40.3)	27.1 (59.7)	45.4
			180	19.4 (24.1)	61.2 (75.9)	80.6
		−Serum	42	10.7 (46.3)	12.4 (53.7)	23.1
			180	7.7 (33.0)	15.6 (67.0)	23.3
Valine	Growing	+Serum	42	175.0 (44.1)	221.0 (55.9)	396.0
			82	202.0 (29.3)	488.0 (70.7)	690.0
			180	195.0 (7.0)	954.0 (83.0)	1,149.0
		−Serum	42	156.0 (38.2)	252.0 (61.8)	408.0
			82	105.0 (26.3)	294.0 (73.7)	399.0
			180	57.5 (14.0)	352.0 (86.0)	409.5
	Nongrowing (hyperconfluent)	+Serum	42	41.4 (43.6)	53.5 (56.4)	94.9
			82	48.5 (29.7)	115.0 (70.3)	163.5
		−Serum	42	39.2 (42.8)	52.3 (57.2)	91.5
			82	32.9 (31.4)	71.7 (68.6)	104.6
			180	24.2 (23.9)	76.9 (76.1)	101.1

[a] Replicate cultures of normal CE cells (95 ± 2 µg protein/dish in the case of nonconfluent growing cells and 841 ± 24 µg protein/dish in the case of hyperconfluent nongrowing cells; i.e. about 10^6 and 10^7 cells, respectively) were washed with the serum-free medium and incubated in 2.5 ml of either the original medium removed and stored before washing, or the serum-free medium, with ^{3}H-arginine (26.4×10^6 cpm) or ^{3}H-valine (44.0×10^6 cpm) for the specified period. After incubation, the monolayers were washed and removed from the dish by trypsinization, and the uptake into acid-soluble and acid-insoluble fractions determined as described in the text. A separate petri dish was used for each time point, each amino acid and each cell density. The values in parentheses are percentages on the total uptake given in the last column.

Fig. 2. The time course of uptake of arginine and lysine by normal CE cells and by RSV-transformed CE cells, at low and high cell densities. For details of experimental conditions and notation, *see* Fig. 1

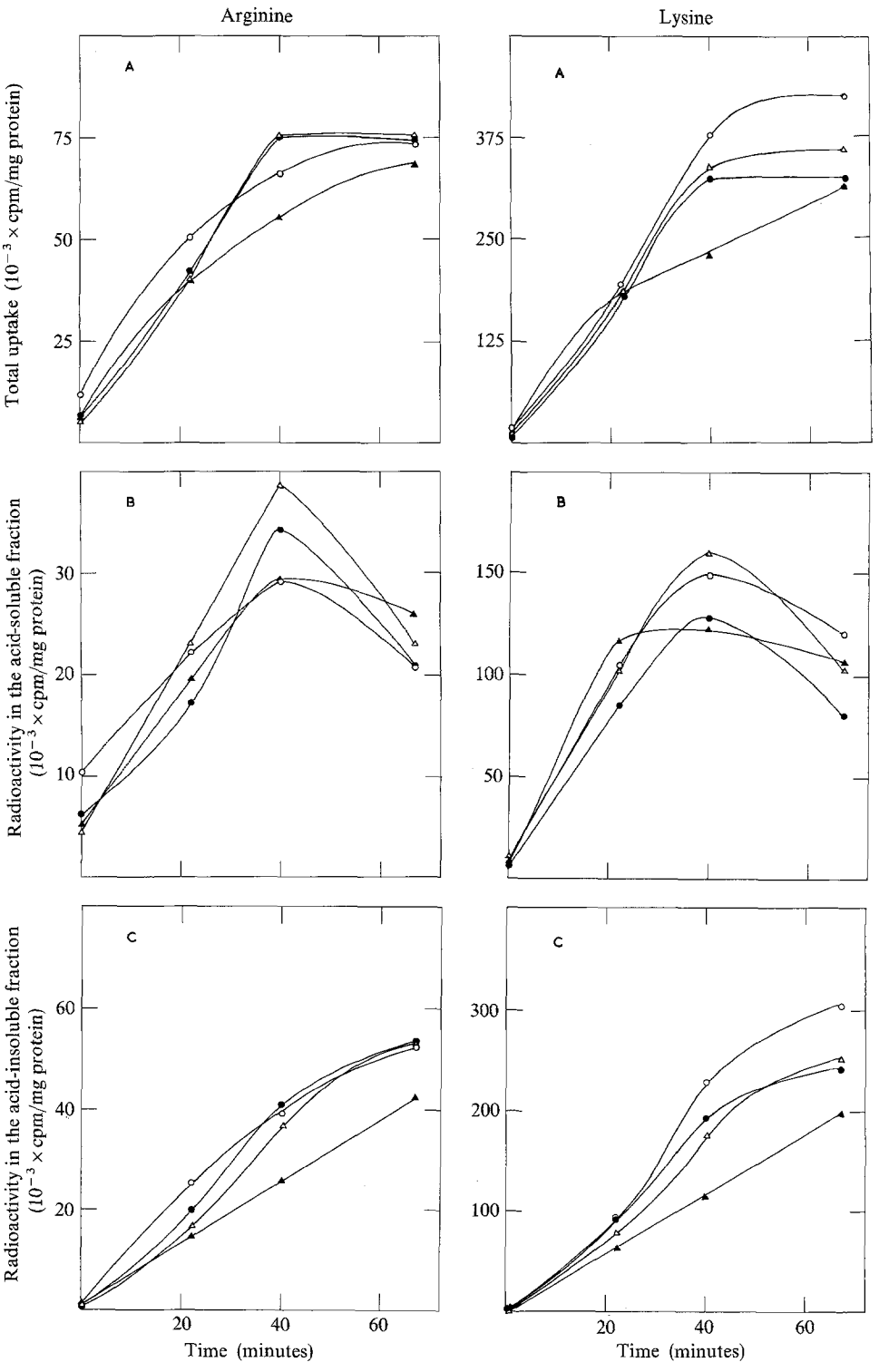
Arginine
Lysine
A
B
C
Total uptake (10^{-3} × cpm/mg protein)
Radioactivity in the acid-soluble fraction (10^{-3} × cpm/mg protein)
Radioactivity in the acid-insoluble fraction (10^{-3} × cpm/mg protein)
75
50
25
375
250
125
30
20
10
150
100
50
60
40
20
300
200
100
20
40
60
Time (minutes)

Fig. 2

beyond 40 min (Table 1); the rate of uptake for the first 40 min in the absence of serum was the same as in the presence of serum for all the amino acids tried.

Effect of Cell Density on the Uptake of Amino Acids

Table 1 shows that the total uptake of arginine and lysine at all time points, both in the absence and in the presence of serum, was four- to fivefold lower in hyperconfluent cultures of CE cells (about 10^7 cells/dish) in which growth was almost completely arrested, than in nonconfluent cultures (about 10^6 cells/dish) in logarithmic growth. The absence of serum from the incubation medium had no effect on the uptake in the first 40 min but, in both types of cultures, no detectable uptake occurred beyond this period in the absence of serum.

The rate of the total uptake of amino acids in nonconfluent cultures containing about 3×10^6 cells/dish was similar to that observed in confluent cultures containing $6–7 \times 10^6$ cells/dish in which cell growth still continued (Figs. 1 and 2). Therefore, during growth of CE cells in culture, the cessation of mitotic activity and a drastic reduction in the rate of uptake of amino acids may occur only at a cell density which is above that at which confluence is achieved.

It may be further noted that the total uptake of arginine and valine for 3×10^6 and 6×10^6 CE cells in Figs. 1 and 2 was about twofold less than that for 10^6 cells in Table 1. This suggests that there may be a continuous decrease in the rate of uptake of amino acids with cell density rather than a sudden decrease beyond a critical cell density. However, this view cannot be taken as established since the experiments in Figs. 1 and 2 on the one hand and in Table 1 on the other, were carried out on different sets of cultures and with different batches of labeled amino acids.

Discussion

Mechanism of Action of Serum

The above experiments strongly suggest that the drastic reduction observed in the rate of the net total uptake of amino acids in growing

normal and RSV-transformed CE cells, 40–50 min after removal of serum is due to a control exercised on the transport of amino acids by some factor(s) contained in serum. This serum factor (SF) or factors may (*a*) exercise a "positive" control on the uptake of amino acids, e.g. by directly stimulating the transport of amino acids into the cells; or (*b*) unblock a "negative" control exercised by a cell factor (CF) on the uptake of amino acid.

The existence of CF is suggested by the observation reported in an accompanying paper [4] that rat liver parenchymal cells in suspension secrete a macromolecular material which is inhibitory to the uptake of amino acids by these cells. Possibility *b* is also supported by the observation that certain proteases mimic the effect of serum in contact-inhibited cultures [7]; these enzymes could act by destroying CF. The present study, however, does not allow an unequivocal choice between the two possibilities.

Effect of Cell Density

In earlier reports, the uptake of phosphate and of nucleic acid precursors (such as uridine), and synthesis of macromolecules, have been shown to proceed at a several-fold higher rate in low-density growing cells than in confluent cells [10, 11, 13, 23, 27, 33–35]. On the other hand, only a marginal difference has been reported between nonconfluent and confluent cells in regard to the uptake of nonmetabolizable amino acids, α-aminoisobutyric acid and cycloleucine [15].

Our data (Table 1) show that in chick embryo fibroblasts, growth stops and the rate of the net uptake of amino acids is drastically reduced when a high cell density (about 10^7 cells/6-cm dish) is reached. As this density is far above the density at which confluency is attained and as no reduction in the rate of uptake was observed on the attainment of confluency (Fig. 1), cell-to-cell contact is presumably not the cause for the decrease in uptake.

A possible cause for the above decrease in the rate of amino acid uptake could be the depletion from the medium of the serum factor (SF) postulated above; addition of fresh serum has been shown to stimulate division of confluent, density-inhibited cells [9, 10, 25, 30]. Alternatively, the postulated serum factor even if not depleted, may not be able to act in hyperconfluent cultures, e.g. due to slower diffusion [28]. Our

data, however, provide only indirect evidence in favor of suggestions such as those stated above. In this connection, it is of significance that the difference we have observed between growing and hyperconfluent nongrowing cells in regard to the rates of uptake of amino acids, is of the same magnitude as the difference in this respect between normal (resting) liver cells and hepatomas (solid and ascitic) [5], or between nondividing and dividing BHK cells in tissue culture [3].

The transport-stimulatory serum factor(s), SF, for which we have presented evidence here, would explain, at least partly, the obligatory requirement for serum exhibited by most cells of higher organisms for growth in tissue culture. The residual uptake of amino acids in hyperconfluent nongrowing cultures, in the presence of serum, could represent the requirement for some protein synthesis during cell maintenance; this low-level uptake must also require a serum factor(s), as it too virtually stopped 40 min after removal of serum. Whether or not this factor is the same as that required for maintenance of the high-level transport in dividing cells, cannot be said at present. Recently, some evidence has been brought forth for the presence of two types of growth factors in serum, one which is necessary for cell multiplication and the other for cell survival [26].

Comparison of Uptake by Normal and RSV-Transformed Cells

In support of earlier observations [19, 20, 23], no significant differences were suggested by our experiments between growing normal and transformed CE cells in regard to the uptake of amino acids in the presence of serum. The continuance of this uptake beyond 40–50 min, however, seemed to be a little less susceptible to the absence of serum in high-density transformed cells than in high-density untransformed cells. The difference between the rates of uptake of amino acids in normal and RSV-transformed cells may be more significant in media containing marginal amounts of serum, as transformed CE cells have a lower requirement of serum than normal cells [29]; the reduction in uptake when hyperconfluency is reached, may also be less marked in the transformed than in the untransformed cells. These points remain to be investigated.

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References

1. Adamson, L.P., Herington, A.G., Bornstein, J. 1972. Evidence for the selection by the membrane transport system of intracellular or extracellular aminoacids for protein synthesis. *Biochim. Biophys. Acta* **282**:352
2. Averdunk, R. 1972. The effect of phytohaemagglutinin and antilymphocyte serum on the transport of potassium ions, glucose and aminoacids in human lymphocytes. *Hoppe-Seyler's Z. Physiol. Chem.* **353**:79
3. Bhargava, P.M., Allin, E.P., Montagnier, L. 1976. Uptake of amino acids and thymidine during the first cell cycle of synchronized hamster cells. *J. Membrane Biol.* **26**:1
4. Bhargava, P.M., Siddiqui, M.A., Kumar, G.K., Prasad, K.S.N. 1975. Effect of cell concentration on the uptake of amino acids by rat liver parenchymal cells in suspension. *J. Membrane Biol.* **22**:357
5. Bhargava, P.M., Szafarz, D., Bornecque, C.A., Zajdela, F. 1976. A comparison of the ability of normal liver, a premalignant liver, a solid hepatoma and the Zajdela ascitic hepatoma, to take up amino acids *in vitro*. *J. Membrane Biol.* **26**:31
6. Biquard, J.M., Vigier, P. 1972. Characteristics of a conditional mutant of Rous sarcoma virus defective in ability to transform cells at high temperature. *Virology* **47**:444
7. Burger, M.M. 1971. Cell surfaces in neoplastic transformation. *Curr. Top. Cell. Regul.* **3**:135
8. Christensen, H.N., Rothwell, J.T., Sears, R.A., Streicher, J.A. 1948. Association between rapid growth and elevated cell concentrations of amino-acids. II. In regenerating liver after partial hepatectomy in the rat. *J. Biol. Chem.* **175**:101
9. Clarke, G.D., Stoker, M.G.P., Ludlow, A., Thornton, M. 1970. Requirement of serum for DNA synthesis in BHK 21 cells; effects of density, suspension and virus transformation. *Nature* **227**:798
10. Colby, C., Rubin, H. 1969. Growth and nucleic acid synthesis in normal cells and cells infected with Rous sarcoma virus. *J. Nat. Cancer Inst.* **43**:437
11. Cunningham, D.D. 1972. Changes in phospholipid turnover following growth of 3T3 mouse cells to confluency. *J. Biol. Chem.* **247**:2464
12. Cunningham, D.D., Pardee, A.B. 1969. Transport changes rapidly initiated by serum addition to "contact inhibited" 3T3 cells. *Proc. Nat. Acad. Sci. USA* **64**:1049
13. Cunningham, D.D., Pardee, A.B. 1971. Transport and phospholipid change related to control of growth in 3T3 cells. *In:* Growth Control in Cell Cultures. G.E.W. Wolstenholme and J. Knight, editors. p. 207. Churchill Livingstone, London
14. Foster, D.O., Pardee, A.B. 1969. Transport of aminoacids by confluent and non-confluent 3T3 and polyoma virus-transformed 3T3 cells growing on glass cover slips. *J. Biol. Chem.* **244**:2675
15. Golde, A., Vigier, P. 1961. Growth of Rous sarcoma virus and cells in non-confluent chick embryo monolayers. *Virology* **15**:36
16. Golde, A., Villaudy, J. 1972. The effect of ageing and cell density on the infection and the morphological conversion *in vitro* by Rous sarcoma virus of chick embryo fibroblasts. *In:* The Biology of Oncogenic Viruses, Proc. 2nd Lepetit Colloquium, Paris, 1972. L. Silvestri, editor. p. 124. North-Holland Publ. Co., Amsterdam
17. Goldfine, I.D., Gardner, J.D., Neville, D.M. 1972. Insulin action in isolated rat thymocytes. *J. Biol. Chem.* **247**:6919
18. Griffiths, J.B. 1972. Role of serum, insulin and aminoacid concentration in contact inhibition of growth of human cells in culture. *Exp. Cell. Res.* **75**:47
19. Hare, J.D. 1967. Location and characteristics of the phenylalanine transport mechanism in normal and polyoma-transformed hamster cells. *Cancer Res.* **27**:2357
20. Hare, J.D. 1970. Quantitative aspects of thymidine uptake into the acid-soluble pool of normal and polyoma-transformed hamster cells. *Cancer Res.* **30**:684

21. Hare, J.D. 1972. A labile, serum-dependent uridine uptake function in mouse embryo cells. *Biochim. Biophys. Acta* **282**:401
22. Hatanaka, M., Hanafusa, H. 1970. Analysis of a functional change in membrane in the process of cell transformation by Rous sarcoma virus; alteration in the characteristics of sugar transport. *Virology* **41**:647
23. Hershko, A., Mamont, P., Shields, R., Tomkims, G.M. 1971. Hypothesis relating growth regulation in mammalian cells to stringent controls in bacteria. *Nature, New Biol.* **232**:206
24. Holley, R.W., Kiernan, J.A. 1968. Contact inhibition of cell division in 3T3 cells. *Proc. Nat. Acad. Sci. USA* **60**:300
25. Kay, J.E. 1972. Lymphocyte stimulation by phytohaemagglutinin: Role of the early stimulation of potassium uptake. *Exp. Cell. Res.* **71**:245
26. Leffert, H.L., Paul, D. 1973. Serum dependent growth of primary cultured differentiated foetal rat hepatocytes in arginine-deficient medium. *J. Cell. Physiol.* **81**:113
27. Levine, E.M., Becker, Y., Boone, C.W., Eagle, H. 1965. Contact inhibition, macromolecular synthesis and polyribosomes in cultured diploid fibroblasts. *Proc. Nat. Acad. Sci. USA* **53**:350
28. Stoker, M.G.P. 1973. Role of diffusion boundary layer in contact inhibition of growth. *Nature* **246**:200
29. Temin, H.M. 1966. Studies on carcinogenesis by avian sarcoma viruses. III. The differential effect of serum and polyanions on multiplication of uninfected and converted cells. *J. Nat. Cancer Inst.* **37**:167
30. Temin, H.M., Pierson, R.W., Jr., Dulak, J.C. 1972. The role of serum in the control of multiplication of avian and mammalian cells in culture. *In:* Growth, Nutrition and Metabolism of Cells in Culture. G.H. Rothblat and J.J. Cristofalo, editors. p. 50. Academic Press, New York & London
31. VanDenBerg, K.J., Betel, I. 1973. Increased transport of 2-aminobutyric acid in rat lymphocytes stimulated with Concanavalin A. *Exp. Cell. Res.* **76**:63
32. VanDenBerg, K.J., Betel, I. 1973. Selective early activation of a sodium-dependent aminoacid transport system in stimulated rat lymphocytes. *FEBS Lett.* **29**:149
33. Waller, J.M., Kirsten, W.M. 1970. Density-dependent inhibition of protein synthesis in normal and virus-transformed cells. *Virchows Arch.* **6**:183
34. Weber, M.J., Edlin, G. 1971. Phosphate transport, nucleotide pools and ribonucleic acid synthesis in growing and in density-inhibited 3T3 cells. *J. Biol. Chem.* **246**:1828
35. Weber, M.J., Rubin, H. 1971. Uridine transport and RNA synthesis in growing and in density-inhibited animal cells. *J. Cell. Physiol.* **77**:157
36. Whitney, R.B., Sutherland, R.M. 1973. Effects of chelating agents on the initial interaction of phytohaemagglutinin with lymphocytes and the subsequent stimulation of aminoacid uptake. *Biochim. Biophys. Acta* **228**:790