JOURNAL OF CELLULAR PHYSIOLOGY 110:29-34 (1982)

Membrane Transport Properties Differ Following Return of Serum-Deprived Versus Ca^{++}-Deprived Human Fibroblasts to a Proliferative State

JOSEPH T. TUPPER,* WILLIAM T. RYALS, AND PETER V. BODINE
Department of Biology, Syracuse University, Syracuse, New York 13210

Human lung fibroblasts (WI38) can be brought to a quiescent state by removal of serum from the medium or by lowering of the extracellular Ca^{++}. Upon return of Ca^{++} or serum, the cells enter the G1 phase and progress to S within 15–18 hours. Since multiple G1 phase blocks have been demonstrated, we wished to determine whether the Ca^{++} and serum block were equivalent since previous data suggested that these two medium components may act at a common point in the initiation of proliferation. We have evaluated the membrane transport of ^{86}Rb, 3-0-methylglucose, AIB, and cycloleucine following stimulation of quiescent cells by Ca^{++} or serum. Serum stimulation results in large increases in the influx of all the substances tested. These increases are prevented if Ca^{++} is absent upon serum stimulation or they are rapidly diminished following Ca^{++} removal. In contrast, Ca^{++} stimulation of Ca^{++}-deprived cells causes little or no enhancement of any of the transport systems, yet the cells progress to S phase in a manner similar to serum-stimulated cells. These results indicate that the Ca^{++} and serum G0 or G1 block are not equivalent and that the serum-induced change in transport of these components does not appear necessary for successful G1 phase progression. Furthermore, the data suggest that the sequence in which Ca^{++} or serum are presented to the cells alters the ability of Ca^{++} to modulate the transport systems. Quiescent cells which are exposed to Ca^{++} prior to serum possess a Ca^{++} modulation of several transport systems. Cells which are exposed to Ca^{++} subsequent to serum do not appear to possess this Ca^{++} regulation.

Under numerous growth-restrictive conditions normal cells in in vitro culture will become quiescent, generally in the early G1 or G0 phase of the cell cycle. Previous reports indicate that exposure of human diploid fibroblasts to suboptimal levels of serum or Ca^{++} for several days brings the cells to a quiescent state (e.g., Hazelton et al., 1979; Tupper et al., 1980). The cells appear to be blocked at a similar point in early G1 phase since, upon supplementation of the culture medium with serum or Ca^{++}, the cells progress to S phase with a similar 15–18-hour lag period (Tupper et al., 1980). Previous observations suggest that Ca^{++} and serum or specific growth factors contained in serum may act in a synergistic manner during initiation of the growth response (e.g., Dulbecco and Elkington, 1975; McKeehan and McKeehan, 1979). Our previous data indicate that serum-induced entrance of WI38 cells to the G1 phase is dependent on the presence of Ca^{++} for the first few hours following serum stimulation (Tupper et al., 1980). Since both serum and Ca^{++} are required for entrance to G1 phase and since either serum-deprived or Ca^{++}-deprived cells, upon stimulation by the appropriate agent, require a similar time period to progress to the G1 phase, we wished to evaluate whether certain aspects of the pleiotypic response (Hershko et al., 1971) were equivalent upon growth initiation from these apparently similar quiescent states. A similar response of the cells to the two growth stimuli would suggest that the cells were blocked in a manner which was not only temporally but biochemically related. This in turn would focus on the mechanism by which Ca^{++} and serum might commonly regulate normal cell growth and why this mechanism is relaxed in transformed cells (e.g., Holley and Keirnan, 1971; Balk et al., 1973). The nature of the Ca^{++} versus serum G1 block is also of interest in view of previous evidence which suggests that, dependent on the particular components of the plasma or growth media, cells can selectively be blocked at different points in the G1 phase (e.g., Burstin et al., 1970; Martin and Stein, 1976; Stiles et al., 1979).

Received April 21, 1981; accepted June 5, 1981.

*To whom reprint requests/correspondence should be addressed.

0021-9541/82/1101-0029$02.00

We have chosen to evaluate the response of the cells to Ca^{++} or serum stimulation by monitoring changes in membrane transport activity, a component of the pleiotypic response in various cells. The unidirectional influxes of Rb^{+}, an analogue for K^{+} transport, 3-0 methyl glucose, cycloleucine, and α-aminoisobutyric acid have been monitored following stimulation by Ca^{++} or serum. As in several other cell types, substantial increases in the transport of these substances occurs following serum stimulation of serum-deprived cells. Furthermore, the absence of Ca^{++} at the time of serum stimulation inhibits these increases or rapidly diminishes them upon removal at later period in G1 phase. In contrast, Ca^{++} stimulation of Ca^{++}-deprived cells has little or no effect on the various transport systems see Christensen, 1970).

METHODS

The methods for serial cultivation of the WI38 human lung fibroblast and the SV40 transformed counterpart have been described in detail previously (Hazelton et al., 1979; Tupper et al., 1980). Manipulation of culture medium divalent cation content, methods for obtaining quiescent cell cultures, and techniques for thymidine incorporation and autoradiography have also been previously published (e.g., Tupper et al., 1980). Cell counts were determined using an electronic cell counter and protein was measured using a Bio-Rad colorimetric assay (Bradford, 1976).

Cells to be used for transport assays were plated at 1-2 $\times 10^5$ cells/plate in 35-mm plastic dishes. At confluency the cells were washed once with Ca^{++}-Mg^{++} free Hanks' balanced salts and incubated another 4 days in serum-free basal diploid medium-Eagle (BME) or in low Ca^{++} BME (0.02 to 0.04 mM Ca^{++}) prepared as described previously (Hazelton et al., 1979). The quiescent cells were then stimulated to enter the G1 phase by addition of 10% fetal bovine serum (FBS) in fresh BME, 10% FBS only, 2 mM $CaCl_2$ in fresh BME, or 2 mM $CaCl_2$ alone, depending on the experiment in question.

Unidirectional influx of alpha-aminoisobutyric acid (AIB), and 1-aminocyclopentane carboxylic acid (cycloleucine) were measured from the kinetics of ^{3}H-AIB or ^{14}C-cycloleucine uptake, the linearity of which was determined in preliminary experiments (see Results). At appropriate times following the growth stimulus or prior to it, the volume of the medium in the plates was reduced from 2 ml to 250 μl and trace amounts of the ^{3}H-AIB and ^{14}C-cycloleucine were added. The cells were pulsed for 15 minutes, the medium was aspirated, and the plates were washed three times with 2 ml of 200 mM choline chloride, 10 mM Tris base adjusted to pH 7.4 with HCl. The cells were then extracted in 1 ml of 0.1 N NaOH for 30 minutes and then 800 μl was removed for assay of isotope in a liquid scintillation counter. The aliquot was counted in 10 ml of Instabray using a dual channel mode for ^{3}H and ^{14}C. The remaining 200 μl was used for protein analysis.

Unidirectional influx of K^{+} was monitored using ^{86}Rb as an analogue for K^{+} movements, the reliability of which has been established for total and ouabain-sensitive K influx (unpublished observations). ^{86}Rb was added in trace amounts with or without 10^{-4} M ouabain [a concentration which maximally inhibits the (Na^{+} + K^{+}) activated ATPase in these cells; [unpublished observations]. The cells were pulsed for 2 minutes and then washed and aliquoted as described above. ^{86}Rb content was measured by detection of Cerenkov radiation in a liquid scintillation counter.

As has been observed in other fibroblasts (e.g., Kletzien and Purdue, 1974; Thrash and Cunningham, 1974), the uptake of 3-0-methyl-glucose (which is neither phosphorylated nor metabolized by the cell) is extremely rapid at 37°C in the WI38 cell. We were unable to obtain initial rates at this temperature. However, by reducing the temperature to 4°C and by assaying ^{3}H-3-0-methyl-glucose uptake in Hanks' balanced salts with glucose adjusted to 10 μM we were able to measure initial rates over a 6-minute incubation period. The cells were washed as above with choline chloride at 0-4°C. Addition of 0.1 mM phloretin to the wash solution did not enhance retention of isotope under these conditions. Cell extracts were aliquoted as above and counted in Instabray.

RESULTS

Figure 1 illustrates the unidirectional influx for the four substances whose transport was evaluated as a measure of the nature of the pleiotypic response following Ca^{++} or serum stimulation. With the exception of 3-0-methyl-glucose, all transport assays were done in complete BME with or without Ca^{++} or serum, as individual experiments dictated. In order to obtain initial rates for the 3-0-methyl-glucose uptake it was necessary to alter the culture conditions as described above. Thus, this data should be viewed with these alterations in mind. Also, it is assumed that changes in unidirectional influx of AIB and cycloleucine are representative of changes in the Na^{+}-dependent A system and the Na^{+}-independent L-amino acid transport system, respectively. However, these analogues are not transported exclusively by these systems such that changes in, for example, the ASC system could contribute to observed changes in influx (for a review on these transport systems see Christensen, 1972).

Figure 2A and B illustrate the amino acid transport response of Ca^{++}-deprived or serum-deprived cells to the addition of Ca^{++} or serum respectively. The cells were brought to quiescence by a 4-day exposure to serum-free medium (containing normal Ca^{++}) or by an equivalent exposure to low Ca^{++} medium (0.02 to 0.04 mM) containing 10% FBS. It is important to point out that at this low Ca^{++} level in the presence of 10% FBS the cells remain firmly attached, viable, and capable of entering the growth cycle upon return of normal Ca^{++} levels. This is not the case if the medium is made "Ca^{++} free" by addition of excess Ca^{++} chelator, such as EGTA, which results in altered cell morphology and detachment after a period of time.

Serum stimulation results in a large and rapid increase in Na-dependent amino acid transport, as evidenced by a nearly threefold increase in AIB influx 1 hour after serum addition. This response is typical of various cell types (for a review see Pardee et al., 1978) including another human diploid fibroblast (Villereal and Cook, 1978). If the cells are serum stimulated using low Ca^{++} medium, the enhanced AIB influx is blocked. Furthermore, replacement of fresh serum medium with fresh serum, low Ca^{++} medium later in the G1 phase results in a reversal of the enhanced uptake. Thus, Ca^{++} has a distinct regulatory effect on the ability of serum or specific growth factors in serum to enhance Na-dependent amino acid transport of serum-deprived, quiescent cells. In contrast, Ca^{++} does not enhance AIB uptake, following Ca^{++} addition to Ca^{++}-deprived cells (Fig. 2A). Thus, it is not simply the presence or absence of Ca^{++} in the medium which regulates the ability of serum to stimulate AIB uptake, since serum containing medium is present upon Ca^{++} addition to the Ca^{++}-deprived cells. One possible

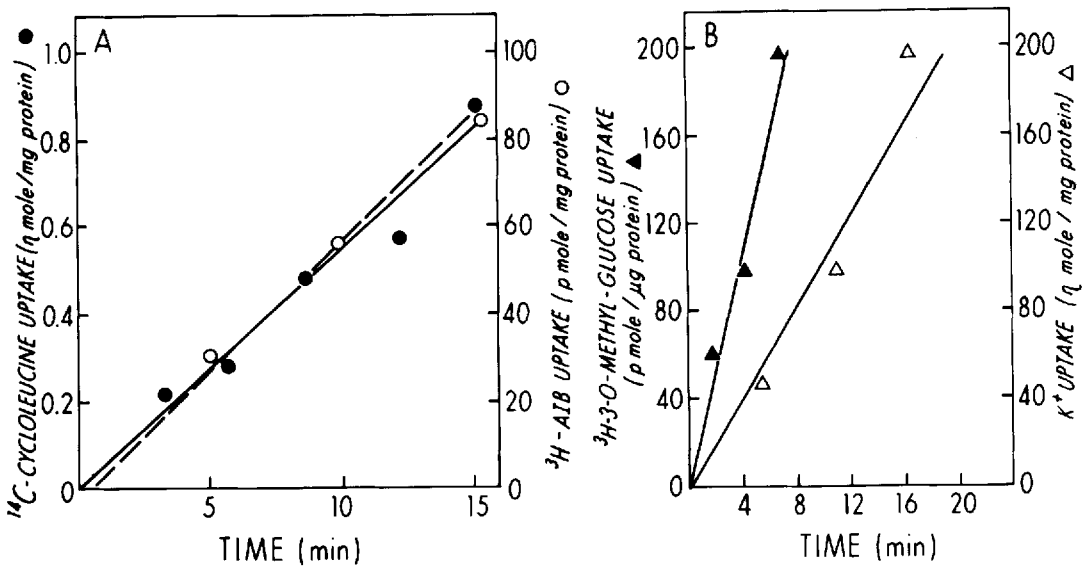

Fig. 1. Initial rates of uptake of AIB, cycloleucine, 3-0-methyl-glucose, and K^+ by the WI38 cell. Assays were performed as described in Methods. All measurements were done on confluent cell cultures in BME containing 10% fetal bovine serum at 37°C with the exception of the 3-0-methyl-glucose uptake which was measured in Hanks' balanced salts at 4°C with glucose adjusted to 10 μM. ^{14}C-cycloleucine was present at 0.1 mM (40 mC/mmol), ^{3}H-AIB at 0.5 μM (25 C/mmol), 3-0-methyl-glucose at 0.7 μM (25 C/mmol), and ^{86}Rb at a trace level with K^+ at 6 mM. Data are mean of duplicate samples.

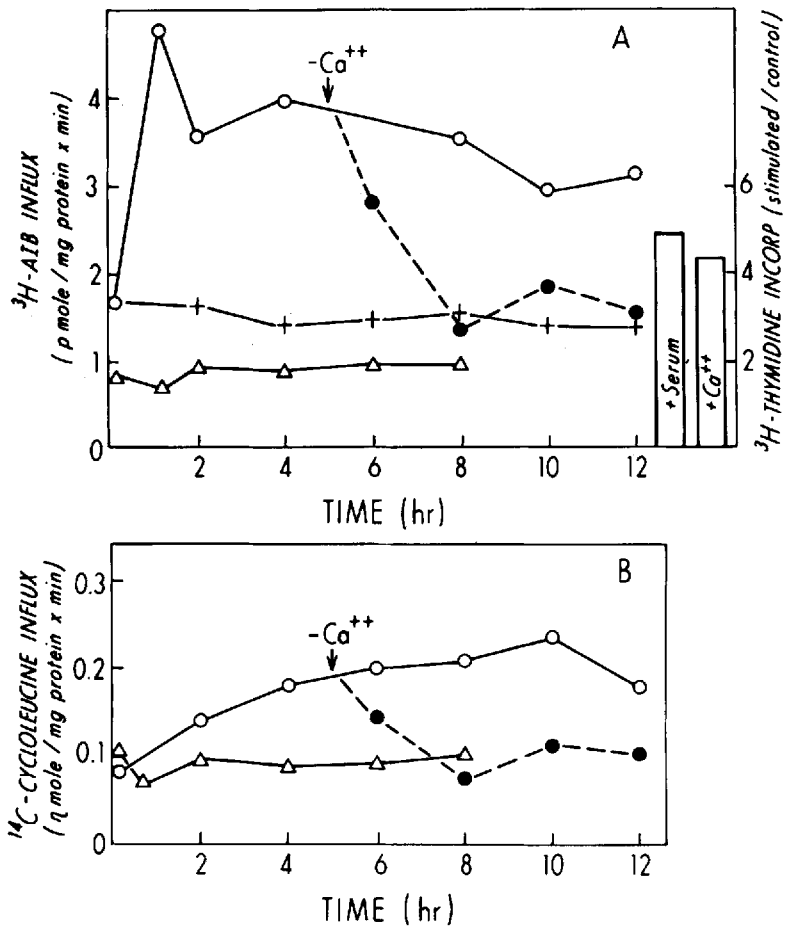

Fig. 2. Unidirectional influx of AIB or cycloleucine following serum stimulation of serum-deprived cells or Ca^{++} stimulation of Ca^{++}-deprived WI38 cells. (A) Cultures were plated as described in Methods and subsequently exposed to serum-free BME or low Ca^{++} BME (approximately 0.4 mM Ca^{++}) for 4 days. The cultures were then stimulated to proliferate by addition of 10% FBS or 1.8 mM $CaCl_2$ at zero hour. The unidirectional influx of AIB was then monitored by pulsing the cells at the times indicated for 15 minutes with ^{3}H-AIB, as in Figure 1A. The points at zero time represent no medium change. The symbols refer to the following conditions: serum-deprived cultures – ○, 10% FBS; + 10%, FBS, low Ca^{++}; ●, 10% FBS, low Ca^{++} at 5 hours; Ca^{++}-deprived cultures – △, 1.8 mM Ca^{++}. (B) The effects of serum or Ca^{++} stimulation on the unidirectional influx of ^{14}C-cycloleucine. This was a double label experiment with panel A, above, and the cells were processed identically. The symbols refer to the following conditions: serum-deprived cultures – ○, 10% FBS; ● 10% FBS, low Ca^{++} at 5 hours; Ca^{++}-deprived cultures – △, 1.8 mM Ca^{++}. In a parallel experiment plates which had or had not been serum stimulated versus plates which had or had not been Ca^{++} stimulated were pulsed at 24 hours after addition of the appropriate stimulus with ^{3}H-thymidine for 1 hour. The ratio of the acid-precipitable radioactivity in stimulated versus control cultures for the serum- or Ca^{++}-activated cells is presented in the right portion of panel A. All data are the mean of duplicate samples.

explanation for this contrasting effect is that the sequence in which the cell receives or loses the necessary growth component is important to the subsequent transport response (See Discussion).

The unidirectional influx of cycloleucine is also enhanced following serum stimulation of serum-deprived cells. The response is substantially slower than observed for AIB, a result in agreement with a previous study on serum-stimulated cycloleucine uptake in the WI38 cell (Costlow and Baserga, 1973). The magnitude of the increase is also substantially less than for the Na-dependent system. This is not unusual for L-system substrates, which in certain cells show little or no enhancement following serum stimulation (e.g., Pardee et al, 1978). The transport of cycloleucine illustrates a similar sensitivity or lack of sensitivity to Ca^{++}, dependent on the mechanism by which quiescence is achieved. A change to serum-containing, low-Ca^{++} medium several hours after serum stimulation reverses the enhanced cycloleucine uptake whereas Ca^{++} addition to Ca^{++}-deprived cells in the presence of serum has no effect on cycloleucine uptake (Fig. 2B).

Figure 2A also illustrates the degree of ^{3}H-thymidine incorporation into acid-precipitable material following a 2-hour pulse given 24 hours after Ca^{++} or serum stimulation of Ca^{++} or serum-deprived cells. The ratio of incorporation in quiescent versus stimulated cells is similar using either method of stimulation, indicating under both conditions the cells enter G1 phase and progress to S phase. This is also consistent with our previous data which monitored the time course of entry to the S phase and the fraction of ^{3}H-thymidine-labeled nuclei, both of which were similar following exit from Ca^{++} or serum deprivation (Tupper et al., 1980).

The data of Figure 2 also indicate the basal rates of AIB or cycloleucine transport are similar in Ca^{++} or serum-deprived cells. This indicates that the absence of a Ca^{++} stimulation of amino acid transport in Ca^{++}-deprived cells does not result from the transport already occurring at a higher rate as compared to serum-deprived cells. However, since these comparisons involved cells from different platings and at slightly different cell densities we felt it appropriate to evaluate basal versus stimulated rates under both conditions of obtaining quiescence in a single experiment. These data are presented in Figure 3. Cells were plated from the same stock at similar densities, serum or Ca^{++} deprived simultaneously 3 days later, and serum or Ca^{++} stimulated 4 days beyond this. We also wished to evaluate the effect of medium changes alone, since certain experimental protocols involved simultaneous changes of medium upon addition or removal of Ca^{++} or serum. As seen in Figure 3, there was no significant difference in the unidirectional influx of either amino acid when comparing Ca^{++} versus serum-deprived cells. Furthermore, when the Ca^{++} or serum were added alone or with fresh BME, the response of the cells was similar. The amount of cell protein under each condition was essentially identical (43μg versus 40 μg/plate). We would also note that we have assayed the amino acid uptake following serum or Ca^{++} stimulation in Hanks' salts with cycloleucine and AIB at 0.1 mM and no other amino acids. The results are similar to those observed in BME with the exception that the absolute magnitude of the fluxes is larger.

Large and rapid increases in K^+ (Rozengurt and Heppel, 1975; Tupper et al., 1977) and glucose transport (Sefton and Rubin, 1971; Kletzien and Perdue, 1974) have also been reported as components of the pleiotypic response. Thus, we wished to ascertain the role of Ca^{++} in these transport changes. The pattern was quite similar to the amino-acid transport changes. Serum initiates large increases in the unidirectional influx of 3-0-methyl-glucose (Fig. 4) and the ouabain-sensitive component of the K^+ influx (Fig. 5). In the case of K^+, the increase is rapidly reversed upon removal of Ca^{++} following serum stimulation. However, Ca^{++} stimulation of Ca^{++}-deprived cells has little effect on the magnitude of the K^+ influx (Fig. 5). The response of 3-0-methyl-glucose was slightly different in that serum stimulation in the absence of Ca^{++} did not entirely block the enhancement of 3-0-methyl-glucose transport but the response was greatly diminished under these conditions (Fig. 4). As with the other transport systems, Ca^{++} failed to enhance 3-0-methyl-glucose influx following Ca^{++} addition to Ca^{++}-deprived cells. Ca^{++} has also been shown to have little effect on 3-0-methyl-glucose transport in chicken fibroblasts (Bowen-Pope and Rubin, 1977).

DISCUSSION

Our previous data indicated that the length of the G1 period was similar following return of either Ca^{++}-deprived or serum-deprived WI38 cells to a proliferative state (Tupper et al., 1980). Other studies have indicated an apparent synergism between Ca^{++} and serum or purified growth factors (e.g., Dulbecco and Elkington, 1975; McKeehan and McKeehan, 1979). The possibility exists that the absence of either serum or Ca^{++} blocks the cells at some common point in the G1 phase. However, previous studies have indicated that cells brought to quiescence by various means are not necessarily in identical physiological states based on the lengths of the G1 phase or differences in membrane transport following release from different blocks (reviewed in Pardee et al., 1978). The present study suggests this to be the case for the Ca^{++} versus serum G1 block in the WI38 cell. Despite the observation that the length of the initial G1 phase following release from either block is similar, the membrane transport response of the cells is very different, indicating they are in distinct physiological states.

The present data also highlight another interesting but as yet unresolved relationship between Ca^{++}, serum, and the regulation of membrane transport. The mechanism by which Ca^{++} deprivation prevents the serum activation of the various transport systems or the mechanism by which Ca^{++} removal subsequently diminishes the serum-induced response is not simply dependent on the presence or absence of Ca^{++} in the culture medium. This is evidenced by the lack of a transport response upon Ca^{++} addition to Ca^{++}-deprived cells. One possible mechanism for this difference in response to Ca^{++} could reside in a specific sequential requirement for serum and Ca^{++} to enhance the transport systems. The current data would indicate that for enhanced transport to occur and to be sensitive to Ca^{++}, the cells must be in a Ca^{++}-containing medium prior to serum activation. The reverse situation, i.e., Ca^{++} addition to Ca^{++}-deprived cells (but in the presence of serum capable of supporting subsequent proliferation), may not provide the appropriate sequence of the two growth regulating substances for generation of the "typical" pleiotypic response. The removal of Ca^{++} from the medium or addition of serum to the medium causes a reduction in membrane-associated Ca^{++} in various cell types (Borle, 1969; Tupper et al., 1978;

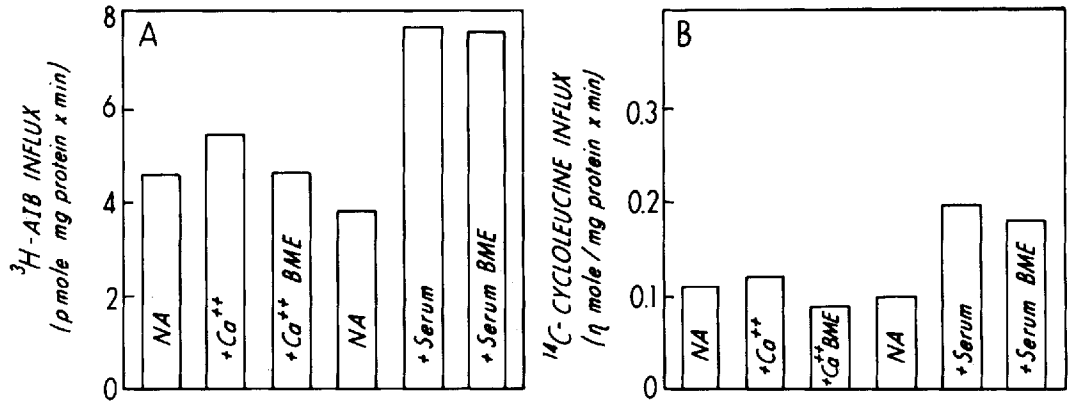

Fig. 3. Comparison of the effects of serum and Ca^{++} stimulation of quiescent cells in the presence or absence of a simultaneous change of culture medium. Cells were brought to quiescence by Ca^{++} or serum deprivation, as in Figure 2. They were then stimulated by the appropriate agent, either by addition of 10% FBS or 1.8 mM $CaCl_2$ alone or these agents in fresh BME. Four hours after stimulation the cells were pulsed with ^{3}H-AIB and ^{14}C-cycloleucine as in Figure 1. NA designates no addition or medium change. BME designates the agent was added in fresh BME after removal of the previous culture medium. Data are mean of duplicate samples.

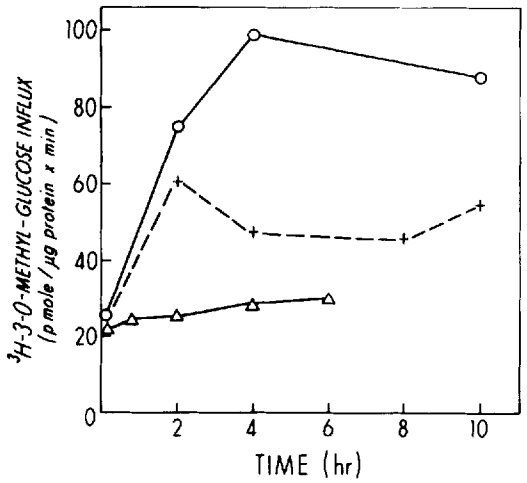

Fig. 4. The effects of serum or Ca^{++} stimulation on the unidirectional influx of 3-0-methyl-glucose. Cells were brought to quiescence by serum or Ca^{++} deprivation for 4 days, as in Figure 2. The cells were then stimulated to proliferate by addition of 10% FBS or 1.8 mM Ca^{++} to serum- or Ca^{++}-deficient cells, respectively. ^{3}H-3-0-methyl-glucose uptake was then measured as in Figure 1B at the times indicated. The symbols are as follows: serum-deprived cells – ○, 10% FBS; +, 10% FBS, low Ca^{++}; Ca^{++}-deprived cells – △, 1.8 mM Ca^{++}. Zero time points represent no medium change. Data are the mean of duplicate samples.

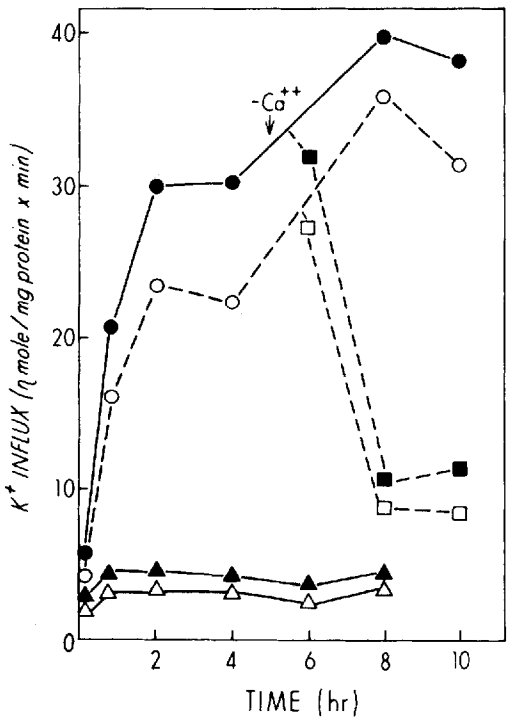

Fig. 5. Serum and Ca^{++} effects on the activation of the ouabain-sensitive, ($Na^+ + K^+$) activated ATPase. Cells were brought to quiescence by serum or Ca^{++} deprivation, as in Figure 2. At zero time they were given 10% FBS or 1.8 mM Ca^{++} and pulsed with ^{86}Rb (an analogue for K^+) for 15 minutes with or without 10^{-4} M ouabain (which was added simultaneously with the ^{86}Rb). Zero time points designate no serum or Ca^{++} addition. The symbols are as follows: serum-deprived cells – ●, 10% FBS, total K^+ influx; ○, 10% FBS, ouabain-sensitive K^+ influx; ■, 10% FBS, $-Ca^{++}$ at 5 hours, total K^+ influx; □ 10% FBS, $-Ca^{++}$ at 5 hours ouabain-sensitive K^+ influx; Ca^{++}-deprived cells – ▲, 1.8 mM Ca^{++}, total K^+ influx; △, 1.8 mM Ca^{++}, ouabain-sensitive K^+ influx. Data are mean of duplicate samples.

Sanui and Rubin, 1979) including the human fibroblast (Villereal, submitted for publication; Tupper, unpublished observations). Thus, it would not be surprising that an appropriate membrane architecture or fluidity, dependent on the degree of membrane-associated Ca^{++}, would be required for the serum-induced transport changes. The absence of Ca^{++} could result in the inability of the specific factor or factors in serum to properly interact with some membrane domain.

The present study indicates that the transport response and the underlying reason for it appears to represent an epiphenomenon as regards control of G1 progression. Basal transport rates of the four substances tested appear to be sufficient for at least one progression of the cells out of quiescence and into the S phase. These data add to an increasing number of reports suggesting that certain changes in membrane transport function following growth activation can be dissociated from regulatory processes in G1 phase. Serum-induced enhancement of 3-0-methyl-glucose uptake and phosphate uptake can be dissociated from successful G1 progression in several cell types (e.g., Greenberg et al., 1977; Thrash and Cunningham, 1974). The level of ($Na^+ + K^+$) activated ATPase activity is equi valent in growing versus serum deprived preconfluent 3T3 cells (Tupper and Zografos, 1978). The serum-induced activation of the ($Na^+ + K^+$) activated ATPase in density-in-

hibited 3T3 cells can be eliminated by low levels of ouabain with no inhibition of G1 phase progression (Frantz et al., 1981). AIB uptake was found to decrease in density-inhibited mouse mammary cells, but testosterone stimulation of growth did not enhance AIB uptake (Robinson and Smith, 1976). There is little fluctuation in the uptake of cycloleucine during cell cycle progression in the Ehrlich ascites cell (Tupper et al., 1976) and no change in L-system transport activity associated with quiescence of the 3T3 cell (Oxender et al., 1977). Thus, for the four transport systems examined in the present study, plus others, there is precedent for the dissociation of transport activity from growth regulation. The ability of the WI38 cell to progress through G1 phase in the absence of a substantial change in the activity of all four of these systems is, therefore, reasonable.

Previous data indicate that serum activates an amiloride-sensitive component of Na^+ influx in 3T3 cells (Smith and Rozengurt, 1978), neuroblastoma cells (Moolenaar et al., 1981), and human fibroblasts (Villereal, 1981). In the human fibroblasts this component of Na^+ influx is activated as well by elevated intracellular Ca^{++} levels or reduced extracellular Ca^{++} levels (Villereal, 1981, submitted for publication). We have previously demonstrated enhanced Na^+ influx in quiescent WI38 cells following Ca^{++} deprivation, an observation consistent with extracellular Ca^{++} regulation of the amiloride-sensitive pathway (Hazelton and Tupper, 1981). It has been suggested that the mitogen-induced enhancement of the Na^+ permeability is a primary regulatory event leading to the initiation of proliferation (Smith and Rozengurt, 1978). The presence of enhanced Na^+ permeability in cells brought to quiescence by Ca^{++} deprivation (Hazelton and Tupper, 1981) indicates that the increased Na^+ influx alone is not growth stimulatory. Recent evidence suggests that the enhanced Na^+ influx following serum stimulation of neuroblastoma cells is electroneutral and possibly represents a Na^+/H^+ exchange mechanism (Moolenaar et al., 1981). It is quite possible that treatments such as Ca^{++} deprivation or serum stimulation, both of which result in displacement of membrane-localized Ca^{++} (Tupper et al., 1977; 1978; Sanui and Rubin, 1979; Villereal, submitted for publication), enhance Na^+ influx by opening a Ca^{++}-regulated membrane gate. However, the two treatments have very different outcomes – growth stimulation or growth repression. Thus, as suggested by Moolenaar et al. (1981), it is what the Na^+ influx is coupled to which may be critical and this may differ depending on the physiological state of the cell.

ACKNOWLEDGMENTS

This work was supported by grants to J.T.T. from the American Cancer Society (BC181B) and the National Institutes of Health (CA17203).

LITERATURE CITED

Balk, S.D., Whitfield, J.F., Youdale, T., and Braun, A.C. (1973) Roles of calcium, serum, plasma and folic acid in the control of proliferation of normal and Rous sarcoma virus infected chicken fibroblasts. Proc. Natl. Acad. Sci. U.S.A., 70: 675-679.

Borle, A.B. (1969) Kinetic analysis of calcium movements in HeLa cell cultures. J. Gen. Physiol., 53:43-56.

Bowen-Pope, D., and Rubin, H. (1977) Magnesium and calcium effects on uptake of hexoses and uridine by chick embryo fibroblasts. Proc. Natl. Acad. Sci. U.S.A., 74: 1585-1589.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Burstin, S.J., Meiss, H.K., and Basilico, C. (1970) Effects of withdrawal of a mitogenic stimulus on progression of fibroblasts into S phase: Differences between serum and purified multiplication-stimulating activity. J. Cell. Physiol., 91: 441-448.

Christensen, H.N. (1970) Linked ion and amino acid transport. In: Membranes and Ion Transport, ed. E.E. Bittar. Wiley, New York, pp. 365-394.

Costlow, M., and Baserga, R. (1973) Changes in membrane transport function in G0 and G1 cells. J. Cell. Physiol., 82: 411-420.

Dulbecco, R., and Elkington, J. (1975) Induction of growth in resting fibroblastic cell cultures by Ca^{++}. Proc. Natl. Acad. Sci. U.S.A., 72: 1584-1588.

Frantz, C.N., Nathan, D.G., and Scher, C.D. (1981) Intracellular univalent cations and regulation of the Balb/c-3T3 cell cycle. J. Cell Biol., 88: 51-56.

Greenburg, D., Barsh, G., Ho, T., and Cunningham, D. (1977) Serum stimulated phosphate uptake and initiation of fibroblast proliferation. J. Cell. Physiol., 90: 193-210.

Hazelton, B., and Tupper, J.T. (1981) Intracellular ionic changes in normal and transformed human fibroblasts after extracellular Ca^{++} deprivation. Biochem. J., 194: 707-711.

Hazelton, B., Mitchell, B., and Tupper, J. (1979) Calcium, magnesium, and growth control in the WI-38 human fibroblast cell. J. Cell Biol., 83: 487-498.

Hershko, A., Mamont, P., Shields, R., and Tomkins, G. (1971) Pleiotypic response. Nature, 232: 206-211.

Holley, R.W., and Kiernan, J.A. (1971) In: Growth Control in Cell Cultures. G.E.W. Wolstenholme and J. Knight, eds. Churchill and Livingstone, London, pp. 3-10..

Kletzien, R., and Perdue, J.F. (1974) Sugar transport in chick embryo fibroblasts. J. Biol. Chem., 249: 3383-3387.

Martin, R.G., and Stein, S. (1976) Resting state in normal and simian virus 40 transformed Chinese hamster lung cells. Proc. Natl. Acad. Sci. U.S.A., 73: 1655-1659.

McKeehan, W.L., and McKeehan, K.A. (1979) Epidermal growth factor modulates extracellular Ca^{++} requirement for multiplication of normal human skin fibroblasts. Exp. Cell Res., 123: 397-400.

Moolenaar, W.H., Mummery, C.L., van der Saag, P.T., and de Laat, S.W. (1981) Rapid ionic events and the initiation of growth in serum-stimulated neuroblastoma cells. Cell, 23: 789-798.

Oxender, D.L., Lee, M., and Cecchini, G. (1977) Regulation of amino acid transport activity and growth rate of animal cells in culture. J. Biol. Chem., 252: 2680-2683.

Pardee, A.B., Dubrow, R., Hamlin, J., and Kletzien, R. (1978) Animal cell cycle. Ann. Rev. Biochem., 47: 715-750.

Robinson, J.H., and Smith, J.A. (1976) Density-dependent regulation of proliferating rate in cultured, androgen-responsive tumor cells. J. Cell. Physiol., 89: 111-122.

Rozengurt, E., and Heppel, L.A. (1975) Serum rapidly stimulates ouabain-sensitive ^{86}Rb influx in quiescent 3T3 cells. Proc. Natl. Acad. Sci. U.S.A., 72: 4492-4495.

Sanui, H., and Rubin, A.H. (1979) Measurement of total, intracellular and surface bound cations in animal cells grown in culture. J. Cell. Physiol., 100: 215-225.

Sefton, B. and Rubin, H. (1971) Stimulation of glucose transport in cultures of density-inhibited chick embryo cells. Proc. Natl. Acad. Sci. U.S.A., 68: 3154-3157.

Smith, J.B., and Rozengurt, E. (1978) Serum stimulates the Na^+-K^+ pump in quiescent fibroblasts by increasing Na^+ entry. Proc. Natl. Acad. Sci. U.S.A., 75: 5560-5564.

Stiles, C.D., Capone, G.T., Sher, C.D., Antoniades, H.N., Van Wyk, J.J., and Pledger, W.J. (1979) Dual control of cell growth by somatomedins and platelet-derived growth factor. Proc. Natl. Acad. Sci. U.S.A., 76: 1279-1283.

Thrash, C., and Cunningham, D. (1974) Dissociation of increased hexose transport from initiation of fibroblast proliferation. Nature, 252: 45-47.

Tupper, J.T., and Zografos, L. (1978) Effect of imposed serum deprivation on the growth of the 3T3 cell: Dissociation from changes in potassium ion transport. Biochem. J., 174: 1063-1065.

Tupper, J.T., Mills, B., and Zorgniotti, F. (1976) Membrane transport in synchronized Ehrlich ascites tumor cells: Uptake of amino acids by the A and L system during the cell cycle. J. Cell. Physiol., 88: 77-88.

Tupper, J.T., Zorgniotti, F., and Mills, B. (1977) Potassium transport and content during G1 and S phase following serum stimulation of 3T3 cells. J. Cell. Physiol., 91: 429-440.

Tupper, J.T., Kaufman, L., and Bodine, P.V. (1980) Related effects of calcium and serum in the G1 phase of the human WI38 fibroblast. J. Cell. Physiol., 104: 97-103.

Villereal, M., and Cook, J.S. (1978) Regulation of active amino acid transport by growth-related changes in membrane potential in a human fibroblast. J. Biol. Chem., 253: 8257-8262.

Villereal, M. (1981) Sodium fluxes in human fibroblasts: Effect of serum, Ca^{++}, and amiloride. J. Cell. Physiol., 107: 359-369.