Biochimica et Biophysica Acta, 467 (1977) 51—64

BBA 77689

DISTINCT PROPERTIES OF URIDINE TRANSPORT SYSTEMS IN GROWING, QUIESCENT AND SERUM-STIMULATED HAMSTER EMBRYO CELLS

Y. EILAM * and O. BIBI

Biophysics Section, Institute of Life Sciences, The Hebrew University, Jerusalem (Israel)

(Received September 21st, 1976)

Summary

The kinetics of uridine uptake in growing, quiescent and serum-activated hamster embryo cells are investigated. The maximum velocity of uridine uptake in growing hamster embryo cells, is lower than in the methylcholanthrene transformed hamster cell line (MCT). This kinetic constant is further reduced in quiescent cells. The K_m values in growing and in quiescent hamster embryo cells, as well as in MCT cells are of the same magnitude.

Distinct alterations in the pattern of inhibition by nitrobenzyl 6-mercaptoinosin (NBMI) are detected as growing hamster embryo cells become quiescent. In quiescent cells the maximum level of inhibition is lower and the apparent K_i value for the inhibition is much higher. These changes are due to the lower apparent K'_m values of NBMI-bound carriers and to the slower rate of formation of the carrier-inhibitor complex. The changes in the kinetic properties of the carriers are partly reversed by serum-activation.

The number of inhibitor binding sites (i.e. nucleoside carriers) does not increase by serum-stimulation of quiescent cells (0.36 and $0.34 \cdot 10^5$ sites/cell in quiescent and serum-stimulated cells, respectively). It is implied that the reduction in uridine transport in quiescent cells is probably due to changes in the turnover of the carriers. These changes may be connected with the observed alterations in the properties of carriers or their immediate environment in quiescent cells.

* Present address: Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel.

Abbreviations: NBMI, 6-((4-nitrobenzyl)mercapto)inosine; *p*-MBS, *p*-hydroxymercuribenzene sulfonic acid (sodium salt); MCT, methylcholanthrene transformed hamster cell line; LD, low density cultures, 1 day after plating; HD, high density cultures, 5 days after plating.

Introduction

Untransformed animal cells in culture display a density-dependent inhibition of growth [1]. The rates of uptake of uridine, P_i, hexoses, as well as of some amino acids into such cells decrease markedly with increasing density of the cultures, reaching a minimum level in quiescent cells [2—5]. Changes in the transport rates obtained in normal cells under different cultural conditions usually reflect changes in the V values, whereas the K_m values of the transport process remained constant [5—9].

Changes in V may result from either a change in the number of carriers per cell or a change in their turnover. The latter process may be due to specific alterations in the carriers, or in their immediate environment, in cells undergoing transition from one state to the other.

An attempt was made to decide between these postulated processes by two different approaches. (1) By experiments aimed at detecting alterations in the properties of the carriers in cells under various cultural conditions. (2) By estimating the number of carriers in density-inhibited and serum-stimulated cells.

The uridine transport system has been selected for the present investigation since a high-affinity inhibitor for this system, nitrobenzyl 6-mercaptoinosine (NBMI), was available. The mechanism of interaction between the inhibitor NBMI and the nucleoside transport systems in the methylcholanthrene transformed hamster cell line (MCT) has been described elsewhere [10].

The results reported in the present work indicate that changes in the transport capacity of hamster-embryo cells, in transition from growing to quiescent state or from quiescent to serum-stimulated state, are accompanied by specific alterations in the kinetic properties of the carriers. Serum stimulation did not involve a change in the number of carriers, but presumably in their turnover.

Materials and Methods

Materials. Tritiated uridine (10 Ci/mmol) was purchased from the Israel Atomic Energy Agency, Nuclear Research Centre, P.O. Box 9001, Beer Sheba. Nytrobenzyl 6-mercaptoinosine was synthesized as described previously [10].

Cell cultures. MCT is a cell line derived from a tumor in golden hamsters after inoculation of 10^6 Ham cells (20-methylcholanthrene-transformed hamster embryo cells, Huberman et al. [11]).

Hamster embryo primary cultures were prepared from 12-day-old fetuses of golden hamster as described by Heidelberger et al. [12]. The experiments were carried out on secondary and tertiary cultures.

The cells were grown in Dulbecco's modified Eagle's medium supplemented with fetal calf serum (5% for MCT and 10% for hamster embryo cells) (Gibco) 10% triptose phosphate, 2 mM glutamine and antibiotics (streptomycin, 400 μg/ml; penicillin 400 units/ml; neomycin 4 μg/ml and kanamycin, 5 μg/ml).

Transport measurements. For transport measurements, cells were plated on 5 cm plastic culture dish (Nunc) at $0.5—1.10^6$ cells per dish and kept in a humidified CO_2 (5%) incubator at 37° C. Immediately before measurements the cells were washed twice with 5 ml phosphate buffered saline at 20° C. For up-

take measurements 1 ml of ^{3}H-labelled nucleosides (1—2 μCi/ml) at defined concentrations was added to each plate and the plates were shaken for 5 min at 20° C. Fluxes were usually performed in duplicate. The uptake was terminated by placing the plates on ice and washing 5 times with 5-ml portions of ice cold phosphate buffered saline. 5% trichloroacetic acid was then added, and the plates incubated for 20 min at 5° C. The trichloroacetic acid soluble fraction was counted in a triton-toluene based scintillation fluid.

Results

The uptake of nucleosides into hamster cells is highly susceptible to inhibition by some alkylated derivatives of 6-mercaptonucleosides [10]. Earlier investigations were carried out on MCT, a transformed cell-line derived from a tumor in golden hamster [11]. The MCT cell-line was selected for studying the mechanism of inhibition by NBMI, mainly because these cultures could attain a relatively high cell density ($5 \cdot 10^5$ cells/cm^2, Fig. 1) without marked changes in the transport properties of the cells (Fig. 2). On the other hand, normal hamster embryo cells were selected for the present study. Cultures of hamster embryo cells have a low saturation density ($1 \cdot 10^5$ cells/cm^2, Fig. 1) and the cells become quiescent after 5—6 days in culture.

A. The kinetic properties of uridine transport in cells in different states

(i) The kinetic constants of uridine transport. The kinetics of uridine uptake in MCT and in hamster embryo cells were compared by measuring the rates of uridine uptake at different substrate concentrations, in cells cultured for 1 or 5 days. One-site kinetics were displayed by MCT cells (up to 200 μM uridine) and by hamster embryo cells (up to 100 μM uridine). At higher substrate concentrations there were some indications of the presence of a nonsaturable fraction.

Results reported in the literature indicate that the transport properties of transformed cells are not affected by the density of the cultures [13]. Indeed,

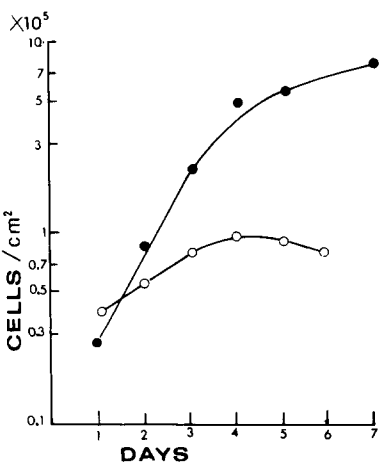

Fig. 1. Growth patterns of hamster-embryo and MCT cultures. Cells were plated at 0.4—0.5 · 10^5 cells/cm^2, and counted daily from 1 to 7 days after plating. ●, MCT cells; ○, hamster embryo cells.

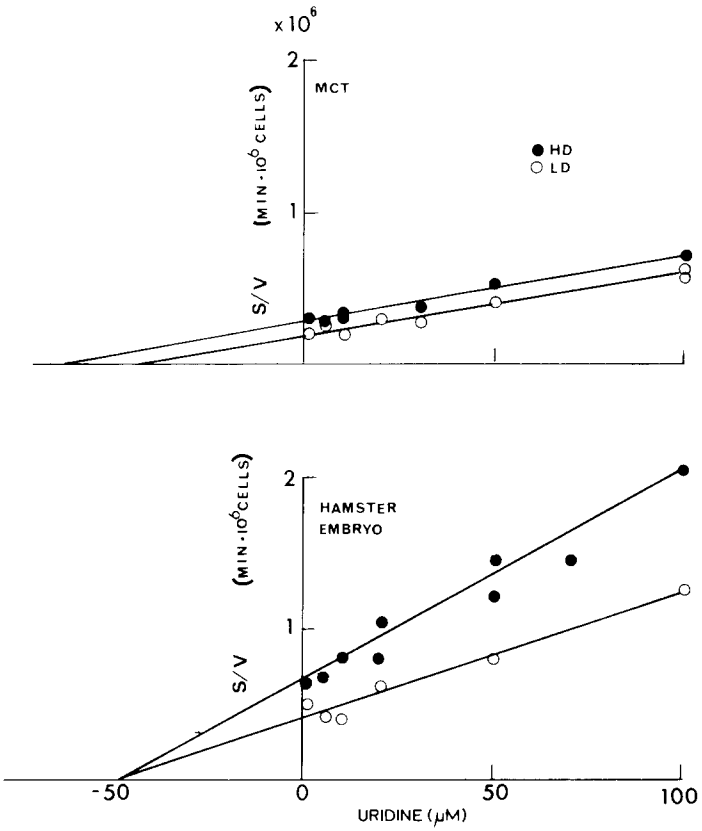

Fig. 2. Kinetics of uridine uptake by MCT and by hamster embryo cells. The rates of uridine uptake were measured at different substrate concentrations after one (LD) or five (HD) days in culture. The numbers of cells per plate were: MCT cells, LD, $1 \cdot 10^6$ cells/plate and HD, $8 \cdot 10^6$ cells/plate. Hamster embryo cells: LD, $0.8 \cdot 10^6$ cells/plate and HD, $1.7 \cdot 10^6$ cells/plate. Kinetic constants given in Table I, were obtained by linear regression.

the maximum velocity of uridine uptake into MCT cells remained constant when sparse MCT cultures became confluent ($8 \cdot 10^6$ cells, Fig. 2 and Table I). On the other hand, under similar conditions, normal hamster embryo cells displayed a considerable decrease in the value of the V (Fig. 2 and Table I). Interestingly, the maximum velocity of uridine uptake into growing hamster embryo cells was much lower than that in growing MCT cells (1.2 and $2.3 \cdot 10^{-10}$ mol/min $\cdot 10^6$ cells respectively, Table I). This difference probably reflects the increased rate of transport in transformed cells [13]. On the other hand, the K_m values displayed by the two types of cells were very similar, both in sparse and in confluent cultures (Fig. 2 and Table I).

(ii) Kinetics of inhibition by NBMI. In order to further investigate the properties of the carriers in cells in different states, we have compared the profile of uridine-uptake inhibition by NBMI in the two types of cells, grown for 1 or for 5 days in culture. The rates of uridine uptake were measured at constant substrate concentration (10 μM) and at varying concentrations of the inhibitor. The results were presented in normalized Dixon plots, with V_0/V_I plotted

TABLE I

KINETIC CONSTANTS OF URIDINE UPTAKE IN MCT AND IN HAMSTER EMBRYO CELLS

Cell type	No. of cells per dish · 10^6	K_m (μM)	V (10^{-10} mol/min · 10^6 cells)
MCT			
1 day after plating	1.0	44 ± 7	2.3
5 days after plating	8.0	61 ± 6	2.3
Hamster embryo			
1 day after plating	0.8	48 ± 13	1.2
5 days after plating	1.7	47 ± 13	0.74

against I; V_0 and V_I are the respective rates of uridine uptake in the absence and in the presence of the inhibitor and I is the inhibitor concentration (Fig. 3).

Similar inhibition profiles were displayed by growing hamster embryo cells (low density) and by MCT cells at low and at high densities. However, the profile of inhibition in hamster-embryo cells changed considerably during transition from growth to quiescence. The maximum level of inhibition decreased from 70 to 50%, and the apparent K_i value increased from 3 nM to 10 nM (Fig. 3). Further changes were observed after an additional day in culture (Fig. 4, lower curve, maximum inhibition level 43%, apparent K_i value 21 nM).

Quiescent hamster embryo cells can be reactivated by incubation, for short periods, in fresh medium supplemented with 20% serum. Such serum activation induced an increase in V but no change in K_m (Fig. 5b and c, lower curves). We investigated whether serum activation would alter the pattern of uridine-transport inhibition by NBMI, as displayed by quiescent cells. It was found that the profile of inhibition obtained after incubation at 37°C with 20% serum resembled that of growing cells (Fig. 4). The maximum level of inhibition increased from 43 to 71% and the apparent K_i value decreased from 21 nM to 1.5 nM.

(iii) The kinetic constants of the NBMI-bound carriers. In order to interpret the reduction in the maximum level of inhibition by NBMI in quiescent cells,

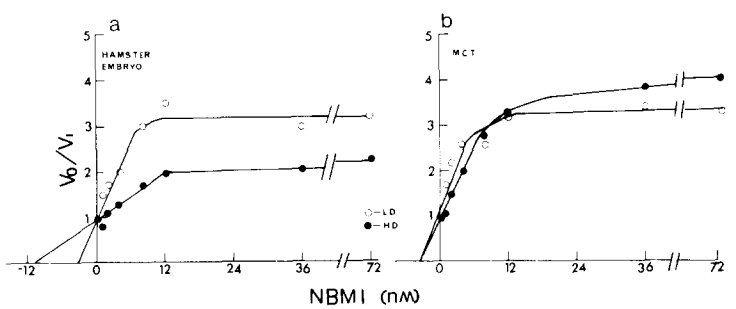

Fig. 3. Pattern of inhibition of uridine uptake by NBMI. Uptake of 10 μM uridine was measured in the presence of different concentrations of NBMI in MCT cells and in hamster embryo cells after 1 day (LD) or 5 days (HD) in culture. V_0 and V_I are the rates of uridine uptake in the absence and in the presence of NBMI respectively.

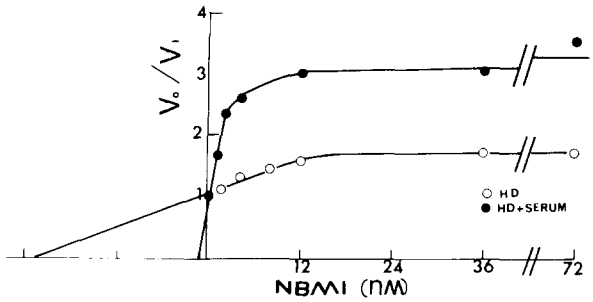

Fig. 4. Pattern of uridine uptake inhibition by NBMI in quiescent and serum activated cells. Hamster embryo cells were grown for 6 days in culture (HD). Serum stimulation was achieved by incubating the cultures with fresh medium, supplemented with 20% dialyzed calf serum, for 1 h at 37°C. V_O and V_I as in Fig. 3.

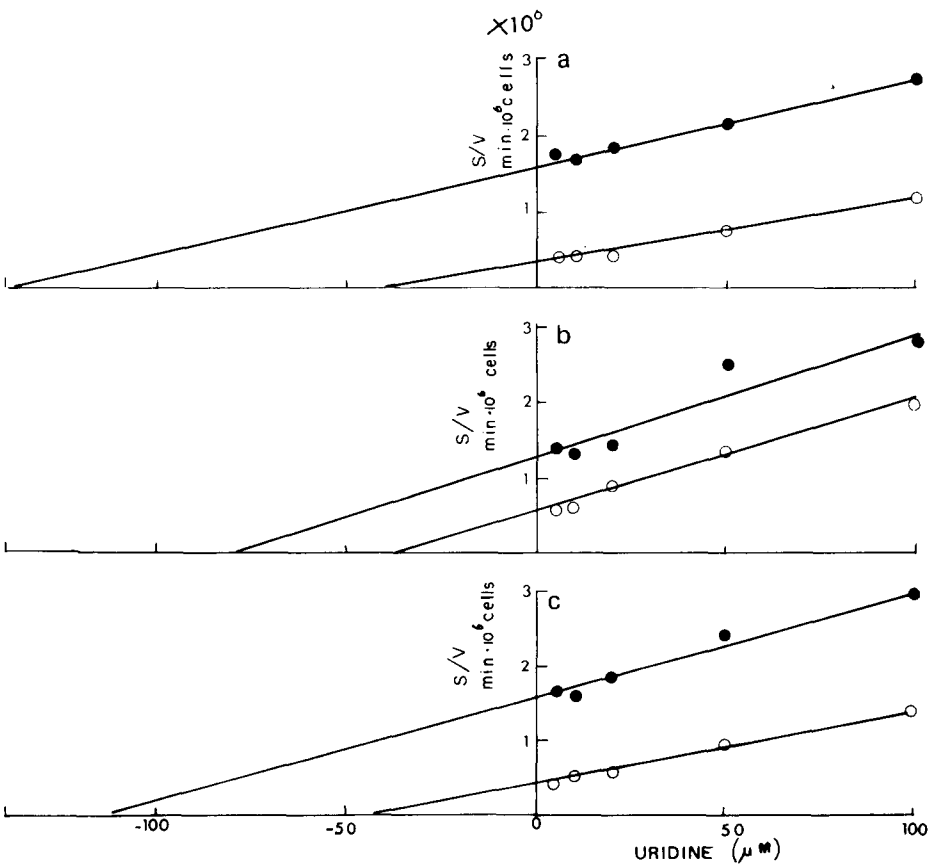

Fig. 5. Kinetics of uridine uptake in the presence of NBMI. Rates of uridine uptake (v) were measured at different concentrations of the substrate (S), (○), in the absence, or (●) in the presence of 20 nM NBMI. Cells were grown for (a) 1 day or (b) 6 days in culture; serum stimulation (c) was achieved as in Fig. 4. Kinetic constants are given in Table II.

TABLE II

KINETIC CONSTANTS OF URIDINE UPTAKE IN THE ABSENCE AND PRESENCE OF NBMI

	NBMI (20 nM)	K_m (μM)	V (10^{-10} mol/min · 10^6 cells)
Growing cells	—	40	1.17
1 day after plating (Fig. 5a)	+	138	0.86
Quiescent cells	—	38	0.69
6 days after plating (Fig. 5b)	+	80	0.62
Serum-activated cells	—	38	1.03
(Fig. 5c)	+	120	0.73

the rates of uridine uptake were measured in growing, quiescent and serum activated cells, at different substrate concentrations, in the presence of a constant concentration of NBMI (20 nM) and in its absence (Fig. 5 and Table II).

In growing cells, NBMI induced a reduction in the V' value from $V = 1.17$ to $V' = 0.86 \cdot 10^{-10}$ mol/min · 10^6 cells and an increase in apparent K_m value from $K_m = 40$ μM to $K'_m = 138$ μM (Fig. 5A and Table II). These results are different from the results previously obtained in MCT cells, where NBMI affected only the K'_m value but not the V' value [10].

In quiescent cells NBMI induced a further reduction in the V' value. But this parameter is also reduced in the absence of NBMI, and the two values of the V are now of the same magnitude ($V = 0.69$ and $V' = 0.62 \cdot 10^{-10}$ mol/min · 10^6 cells) (Fig. 5b and Table II). Interestingly, the K'_m value (apparent K_m, in the presence of the inhibitor) in quiescent cells in considerably lower than in growing cells ($K'_m = 80$ and 138 μM, respectively). All these changes are reversed to some extent by serum activation ($K'_m = 120$ μM, $V' = 0.73$ and $V = 1.03 \cdot 10^{-10}$ mol/min · 10^6 cells) (Fig. 5c and Table II).

The interpretation of the low maximum level of inhibition (I_{max}) in quiescent cells is now simple. At constant substrate concentration, I_{max} can be calculated as follows:

$$I_{max} = 1 - \frac{S \cdot V'/(S + K'_m)}{S \cdot V/(S + K_m)} \quad (1)$$

V' and K'_m are the corresponding parameters of the NBMI-bound carriers. The calculated I_{max} values for 10 μM uridine; 77, 51 and 74% for growing, quiescent and serum activated cells, respectively, accorded well with the results (Figs. 3 and 4).

(iv) The rate constants of the formation of NBMI-carrier complexes. The second feature of the kinetics of inhibition in quiescent cells is the higher apparent K_i value (10—21 nM versus 1—3 nM in growing cells, Figs. 3 and 4). It has previously been shown, in MCT cells, that the rates of formation of the inhibitor-carrier complexes, at low inhibitor concentrations, are time- and temperature-dependent [10]. Therefore, when the inhibitor is present only during a limited period, alterations in apparent K_i values may be due to changes in the rate constant of formation of the inhibitor-carrier complex. To examine this

possibility cultures were preincubated at 20°C for different periods (0.5—10 min), at different concentrations of inhibitor. The cells were immediately flushed with buffer, and uridine uptake was measured. It has previously been found that the inhibition by NBMI was not reversed by such treatment [10]. Since the rate of formation of the inhibitor-carrier complexes can be described by pseudo-first-order kinetics (ln(fractional activity) = $-kIt$, [10]) the logarithms of the fractional activities of uridine uptake into growing, quiescent and serum activated cells were plotted against the duration of preincubation with the inhibitor (Fig. 6). For growing cells, linear plots were obtained, indicating a homogeneous cell population (Fig. 6a). The second order rate constant ($k = 1.6 \cdot 10^8$ min$^{-1} \cdot$ M^{-1}, Fig. 7) was of a magnitude similar to that previously obtained for MCT cells ($k = 1.3 \cdot 10^8$ min$^{-1} \cdot$ M^{-1}, [10].

On the other hand, in quiescent cells the non-linearity of the plots indicated a non-homogeneous cell population (Fig. 6b). It was impossible to analyse the

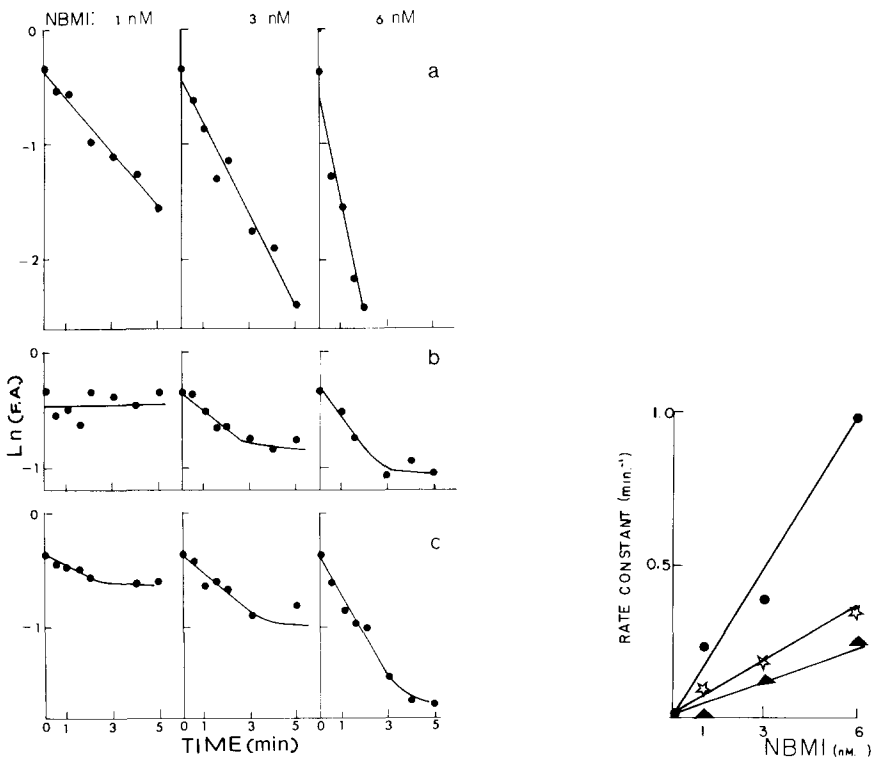

Fig. 6. Rate-constants of the formation of NBMI-carrier complexes. Cultures grown for (a) 1 day; (b) 6 days. (c) serum stimulated 6-day cultures (as in Fig. 4) preincubated with 1, 3 or 6 nM NBMI at 20°C for different periods. Following preincubation, the cultures were immediately flushed with buffer and the rates of uptake of 10 μM uridine were measured. The logarithm of the fractional activities, after subtraction of non-inhibited transport, were plotted against the duration of preincubation.

Fig. 7. The second-order rate constant of NBMI-carrier complex formation. The slopes, obtained by linear regression from the lines in Fig. 6a and from the linear parts of the curves in Fig. 6b and 6c, are plotted against the concentrations of the inhibitor. ●, growing cells (results from Fig. 6a) $k = 1.6 \cdot 10^8$ min$^{-1} \cdot$ M^{-1}; ▲, quiescent cells (results from Fig. 6b) $k = 0.38 \cdot 10^8$ min$^{-1} \cdot$ M^{-1}; ☆, serum activated cells (results from Fig. 6c) $k = 0.62 \cdot 10^8$ min$^{-1} \cdot$ M^{-1}.

rate-components of the curves owing to the large experimental errors. Instead, the apparent rate constants were estimated from the initial slopes of the curves, and replotted against the concentrations (Fig. 7). The second-order rate constant obtained ($k = 0.32 \cdot 10^8$ min$^{-1} \cdot$ M^{-1}, Fig. 7) was much lower than that in growing cells. Serum activation partly reversed the reduction in the rate constant, although the population of the cells still remained nonhomogeneous (Figs. 6c and 7).

The higher apparent k_i value in quiescent cells may be interpreted as due to the marked reduction in the rat constant of inhibitor-carrier interaction.

B. Estimation of the number of uridine carriers

The experiments described in the previous section reveal specific changes in the carriers when growing cells become quiescent. In the present section we attempt a different approach to the analysis of transport regulation.

The activation of quiescent cells by a higher concentration of fresh serum induced in our system an increase in the rate of uridine uptake (10 μM) by approximately 100% (Table IIIA).

As shown in Table IIIA, the presence of NBMI in the medium completely prevents the increase in the rate of transport, in response to serum activation. In order to detect the possible appearance of new transport sites during serum activation, the quiescent cells were allowed first to bind NBMI, and then to be activated by serum. After NBMI-binding the cultures were washed to remove excess inhibitor and were divided into two parts. The previous medium was replaced in one part (Expt. B_1) while fresh medium, supplemented with 20% serum, was added to the other part (Expt. B_2). The cultures were incubated for 20 min at 37°C and the uptake of uridine was measured. The results of the

TABLE III

THE UPTAKE OF URIDINE (10 μM), INTO HAMSTER EMBRYO CELLS IN CONFLUENT CULTURES AFTER PREINCUBATION WITH NBMI AND SERUM ACTIVATION

Cells were grown for 6 days after plating. Cell number, $2 \cdot 10^6$ cells/dish. The medium is that in which the cells were grown for 6 days; the buffer, phosphate buffered saline, pH 7.4.

Preincubation		Uptake of [^{3}H]-uridine (number of counts)
A. 60 min at 37°C		
1. Growth medium		2985
2. Fresh medium + 20% serum		5981
3. NBMI (100 nM) in the growth medium		2075
4. NBMI (100 nM) in fresh medium + 20% serum		2309
B. 5 min at 20°C	20 min at 37°C	
1. NBMI (100 nM) in buffer	growth medium	2787
2. NBMI (100 nM) in buffer	fresh medium + 20% serum	3395
C.		
1. Buffer	growth medium	3003
2. Buffer	fresh medium + 20% serum	4758

experiments (B_2) were compared with the predictions of two models: In model I it is assumed that only carriers free from NBMI respond to the addition of serum by an increase in their turnover. The predicted number of counts for experiment B_2 would be only slightly higher than the number of counts in the control (B_1), since the process of NBMI release from the carriers is time-dependent and proceeds simultaneously with serum activation. In model II it is assumed that serum induces the appearance of carriers, unaffected by the preceding NBMI treatment. The predicted number of counts (Table II) would therefore be, by simple calculation 4542 cpm. The result obtained in experiment B_2 (3395 cpm) is much closer to the predictions of model I. Similar results were obtained in three different experiments.

The results obtained may indicate that serum activation does not induce an increase in the number of carriers. A more direct method to estimate the number of carriers before and after serum activation was attempted by a modification of the bioassay technique used before [10]. Quiescent cells (6 days in culture) were incubated with fresh medium, supplemented with 20% serum, for 1 h at 37°C. This treatment induced a 100% increase in the rate of uridine uptake (Table III). NBMI (24 nM) was then added to plates with quiescent or serum-activated cells. After a short preincubation (15 min at 20°C) the solutions were removed and the cells washed three times with buffer to remove excess and nonspecifically-bound inhibitor. The cells were then incubated with 1 ml buffer for 1 h at 37°C, an incubation period which is sufficient for a complete reversal of the inhibition. The amounts of NBMI released to the buffer solutions were determined by incubating MCT cells in these solutions (1 h at 37°C) and then measuring the fractional inhibition of uridine uptake. The conversion of the fractional inhibition to NBMI concentrations was made with the aid of a calibration curve obtained by incubating MCT culturs in solutions of ten known concentrations of NBMI (0—0.5 nM) followed by measurement of the fractional inhibition of uridine uptake.

The concentrations of NBMI so determined were 0.09 and 0.085 nM, for quiescent and serum activated cells, respectively. The estimated number of inhibitor-binding sites per cell ($2 \cdot 10^6$ cells/plate) were:

$$\text{quiescent cells } \frac{0.09 \cdot 10^{-12} \cdot 6 \cdot 10^{23}}{2 \cdot 10^6} = 0.36 \cdot 10^5 \text{ sites/cell}$$

$$\text{serum activated cells } \frac{0.085 \cdot 10^{-12} \cdot 6 \cdot 10^{23}}{2 \cdot 10^6} = 0.34 \cdot 10^5 \text{ sites/cell.}$$

Discussion

The mechanism leading to the reduction in transport rates in quiescent cells and to the restoration of these rates in serum-stimulated cells is not yet fully understood. It has been established that the culture-dependent alterations in the transport rates result from a change in the V values, — with K_m relatively constant (see ref. 13 for review; Fig. 2 and Table I).

Alterations in the values of the V can be explained by two alternative hypotheses. (a) A change in the number of carriers, while the properties of the carriers remain unchanged. (b) A change in the turnover of the carriers, with a

constant number of carriers. A combination of the two postulated processes is also possible.

The number of carriers may change as a result of a decrease in protein synthesis in quiescent cells. However, since the initial stage of the stimulation of transport by serum does not require protein synthesis de novo [14], it would have to be assumed, for hypothesis (a) that inactive carriers are activated or unmasked, or that previously synthesized carrier-protein is inserted into the membrane. The alternative hypothesis (b), a change in the turnover, would require specific alterations in the properties of the carriers, or in the carriers' immediate environment.

A previous study of the transport of 3-*O*-methyl glucose in BHK cells has indicated that the change in the transport ability of each cell during transition from growth to quiescence and from quiescence to serum stimulation is a discrete and distinct event, whereas the average rate of transport in the cell population changes gradually [15]. A discrete change is more likely to be the result of an alteration in the properties of the carriers, rather than in their number. The present work provides some kinetic evidence in support of hypothesis (b).

The number of ribonucleoside carriers in quiescent and serum activated cells were estimated by the use of the inhibitor NBMI. The high affinity and the specificity of NBMI to ribonucleoside carriers led to the assumption that the number of NBMI binding sites is closely related to the number of ribonucleoside carriers. Using the bioassay procedure we estimated the number of NBMI binding sites per cell to be: quiescent cells, $3.6 \cdot 10^4$ sites/cell; serum activated cells, $3.4 \cdot 10^4$ sites/cell.

These data indicate that serum did not induce an increase in the number of carriers. Therefore, it is concluded that serum activation led to an increase in carrier turnover.

A change in turnover implies that carrier properties change as cells become quiescent. The present study provides evidence that some of the kinetic parameters of uridine carriers were altered as growing hamster embryo cells become quiescent.

Uridine uptake in growing and in quiescent cells differed only in V; the K_m was not significantly altered (Fig. 2 and Table I). Whereas a change in K_m would imply changes in the properties of the carriers, data showing that the K_m is not significantly altered are not sufficient to establish that the carriers remain unchanged. In this case, a more detailed comparison of the properties of the carriers in growing and quiescent cells may be carried out by studying the kinetics of inhibition.

The mechanism of uridine transport inhibition by the specific inhibitor NBMI has previously been investigated in the transformed cell line MCT [10]. The inhibition of uridine transport by NBMI was partial, as 25—30% of transport remained uninhibited even at high inhibitor concentration. In the same study it was indicated that the binding of the inhibitor to the carrier occurred at a site different from the substrate binding site (allosteric binding). Such binding induced conformational changes in the carrier, manifested in the following properties:

(a) The affinity to the substrate decreased from $K_m = 50\ \mu M$, in the absence of NBMI, to $K'_m = 250\ \mu M$ in its presence. V in MCT cells remained unaltered.

(b) Uridine transport was not inhibited by preincubation of the cultures with 20 μM *p*-hydroxymercuribenzenesulfonate (*p*-MBS). However, when uridine transport was measured in the presence of NBMI, the fraction of transport that was not inhibited by NBMI alone was inhibited by the combination of NBMI and *p*-MBS. These results were interpreted as indicating that the two carrier conformations differ in their susceptibility to inhibition by *p*-MBS (for further details see Eilam and Cabantchik [10]).

The comparison between the patterns of uridine transport inhibition by NBMI in MCT cells [10] and in hamster embryo cells, revealed the following similarities. (1) In low density hamster embryo cultures, 30% of transport remained uninhibited at high concentrations of inhibitor (Figs. 3 and 4). (2) As in MCT cells, uridine transport in hamster embryo cells was inhibited by 20 μM *p*-MBS only in the presence of NBMI. No inhibition was observed in the absence of NBMI (Table IV). (3) In low density cultures of hamster embryo cells addition of NBMI induced a decrease in the affinity to the substrate from K_m = 40 μM, in the absence of NBMI, to K'_m = 138 μM in its presence (Fig. 5 and Table II). (4) The rate constants which characterize the formation of the inhibitor-carrier complex, were similar in low density hamster embryo cultures and in MCT cells ($1.6 \cdot 10^8$ and $1.3 \cdot 10^8$ $min^{-1} \cdot M^{-1}$, respectively) (Fig. 7 and ref. 10). (5) The apparent K_i values for the inhibition of uridine transport by NBMI were similar in low density hamster embryo cultures and in MCT cells (3 nM, Fig. 2).

The observations described above led to the conclusion that the mechanism and the kinetic features of uridine transport inhibition by NBMI, in low density cultures of hamster embryo cells, are similar to those previously studied in the transformed cell line MCT [10].

However, in MCT cells, addition of NBMI led to an increased K_m but V unchanged. On the other hand in hamster embryo cells both K_m and V were altered by NBMI (Fig. 5 and Table II). The meaning of this difference has not yet been evaluated.

TABLE IV

THE INHIBITION OF URIDINE UPTAKE BY *p*-MBS AND NBMI IN HAMSTER EMBRYO CELLS, IN DIFFERENT STATES

The cells	Preincubation 30 min at 20° C	Uptake of uridine (10 μM) in the presence of	Fractional activity
Growing cultures			
1 day after plating	buffer *	—	1
	P-MBS (20 μM)	—	0.99
	buffer	NBMI (12 nM)	0.71
	P-MBS (20 μM)	NBMI (12 nM)	0.29
Quiescent cultures			
5 days after plating	buffer	—	1.0
	P-MBS (20 μM)	—	1.38
	buffer	NBMI (12 nM)	0.51
	P-MBS (20 μM)	NBMI (12 nM)	0.16

* Phosphate buffered saline, pH 7.4.

In contrast to the similarity between the profile of uridine transport inhibition by NBMI in MCT cells and in low density hamster embryo cultures, the pattern of inhibition and its kinetic constants changed considerably as growing hamster embryo cells become quiescent (5 days in culture).

(1) The maximum level of inhibition decreased from 70 to 50% (Fig. 2). This decrease was the result of a change in the value of the apparent K'_m measured in the presence of NBMI (K'_m = 138 μM and 80 μM, in growing and quiescent cells, respectively (Fig. 5 and Table II)). (2) The apparent K_i values increased from 3 nM in growing cells to 10 nM in quiescent cells. (3) The rate constant characterizing the formation of the inhibitor carrier complex in quiescent cells was lower than in growing cells ($1.6 \cdot 10^8\ M^{-1} \cdot min^{-1}$ and $0.38 \cdot 10^8\ M^{-1} \cdot min^{-1}$, in growing and quiescent cells, respectively).

The observed differences summarized above indicate that in hamster embryo cells uridine carriers undergo some alterations as growing cells become quiescent. These alterations are partly reversed by addition of serum to quiescent cells (Figs. 4—7). Transition from quiescence to growth, i.e. serum activation, involves an increase in the turnover of carriers, with no change in their number.

The serum-induced increase in the turnover of uridine carriers is here interpreted as the result of specific changes in the carriers or in their immediate environment. Alternatively, such an increase may be due to an increase in the rate of uridine phosphorylation inside the cells. For the latter interpretation to hold, it must be assumed that in quiescent cells the rate of uridine uptake is limited by the rate of phosphorylation. However, the observations summarized below, provide evidence against this assumption.

(a) We have followed the rate of uridine uptake between 20 s and 5 min, in MCT cells (ref. 10) as well as in growing and quiescent hamster embryo cells (Eilam, unpublished). A constant rate was obtained. The experimentally determined zero point coincided with the zero point obtained by extrapolating the linear time course of entry. If phosphorylation was rate limiting initial uptake would have been more rapid than later uptake, which would represent phosphorylation.

(b) Uridine uptake in quiescent cells can be stimulated (30—40%) by preincubating the culture with 20 μM *p*-MBS (Table IV). Similar stimulation is obtained with *p*-hydroxymercuribenzenamidoethyl-Dextran, mol. wt. 10 000 [10]. The latter, which is a macromolecule, reacts only with SH groups localized on the surface of the membrane. It would be difficult to explain its stimulatory effect if phosphorylation had been rate limiting.

(c) Serum stimulation of uridine uptake is completely inhibited by NBMI (Table II), which is known to affect only the transport component of uptake, without affecting the nucleoside phosphorylation [10].

It may, therefore, be assumed that serum stimulation of uridine uptake in quiescent hamster embryo cells (5 days in culture) is due to membranal changes.

Experiments aiming at determining the primary site of serum effects on transport, membrane or metabolism, were often carried out with nonmetabolized analogues. The present results indicate that such a study may lead to erroneous conclusions. We have demonstrated that serum induces an increase in carrier turnover. Since the turnover is usually affected by the particular sub-

strate interacting with the carriers, it is quite possible that serum stimulation of transport might be obtained with one of the substrates which share the same transport system, but not with another.

Acknowledgements

The authors wish to acknowledge J. Schwartz for technical assistance, Dr. E. Huberman (The Weizmann Institute of Science, Rehovot, Israel) for kindly providing the MCP cell-line, Drs. W.D. Stein and I.Z. Cabantchik (Hebrew University, Jerusalem) for stimulating discussions and Dr. I.Z. Cabantchik for kindly supplying NBMI. The work was supported by a NCI contract No. NO1 CP 43307.

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