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INDUCED CHANGES IN THE RATES OF URIDINE-^{3}H UPTAKE AND INCORPORATION DURING THE G_1 AND S PERIODS OF SYNCHRONIZED CHINESE HAMSTER CELLS

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ABSTRACT

The rates of uridine-5-^{3}H incorporation into RNA and the rates of uridine uptake into the acid-soluble pool during the cell cycle of V79 Chinese hamster cells were examined. Cells cultured on Eagle's minimal essential medium supplemented with fetal calf serum, lactalbumin hydrolysate, glutamine, and trypsin displayed rates of incorporation and uptake which increased only slightly during G_1 and accelerated sharply as DNA synthesis commenced. In contrast, cells cultured on minimal essential medium supplemented only with calf serum exhibited rates of incorporation and uptake which increased linearly through both G_1 and S. The transition from one pattern to the other can be induced within 24 hr and is completely reversible. The nonlinear pattern exhibited by cells grown on the supplemented fetal calf serum medium can also be overcome with high exogenous uridine concentrations. In the presence of 200 μM uridine, these cells display a linear pattern of increase in rates of uridine incorporation and uptake. It is concluded that at lower uridine concentrations the pattern of increase in the rate of uridine incorporation into RNA during the cell cycle for a given population of cells is dependent upon the rate of uridine entry into the cell, and that this pattern is not rigidly determined but can be modified by culture conditions.

INTRODUCTION

The progression of cells through the mitotic cycle is characterized by ordered temporal changes in macromolecular synthesis. At metaphase there is no DNA synthesis, protein synthesis is greatly reduced (1), and RNA synthesis appears to be restricted to the synthesis of 4S and 5S RNA (1, 2). The synthesis of DNA is limited to a discrete portion of interphase, whereas proteins as a class of macromolecules appear to be synthesized throughout the cell cycle (3). The synthesis of RNA during the cell cycle has been extensively studied, and the common conclusion has been reached that cells synthesize RNA at all times during interphase, but at a greater over-all rate during the latter part of interphase.

There are conflicting reports regarding the kinetics by which the rates of RNA synthesis increase as cells traverse the cycle. Several incorporation studies (4, 5, 6) have indicated that the rate of RNA synthesis increases only slightly during G_1

and that a marked acceleration occurs at the onset of the S phase. Others (7, 8, 9) have described a linear increase in the rate of RNA synthesis during G_1 and at least the early part of the S period, with no acceleration of the increase in RNA synthetic rate at the time DNA synthesis begins. Seed (10), furthermore, has claimed that rapidly dividing cells from normal tissue exhibit the nonlinear pattern of increase in RNA synthetic rate, whereas cells derived from tumors display a linear increase in RNA synthesis during both the G_1 and S periods. The present study shows that cells may be induced to incorporate uridine-^{3}H into RNA in a manner consistent with either of the above postulated patterns of RNA synthesis, depending upon the constitution of the culture medium. This finding indicates that the pattern of uridine incorporation is not a rigidly defined characteristic of cells. The study also demonstrates that the respective patterns of increase in rate of uridine incorporation into RNA are closely paralleled by the changes in the rate of uridine-^{3}H uptake into the acid-soluble pool. The transition from one pattern of incorporation or uptake to the other is reversible.

MATERIALS AND METHODS

Materials

The thymidine-^{3}H and uridine-5-^{3}H were obtained from Schwarz Bio Research, Orangeburg, N. Y. and Mann Research Labs Inc., New York. Minimal essential medium (MEM) was obtained from Grand Island Biological Co., Berkeley, Calif., and the calf serum and fetal calf serum were purchased from Flow Laboratories, Rockville, Md. and Grand Island Biological Co.

Cell Line and Growth Conditions

A subline of the V79 line of female Chinese hamster lung cells, originally cultured by Ford and Yerganian (11) and kindly provided by Dr. M. Elkind, was used. Stock cultures were grown as monolayers in Blake bottles at 37°C. Two different growth media were used. One was a minimal essential medium (MEM) (12) supplemented with fetal calf serum (15%), lactalbumin hydrolysate (2%), glutamine (0.4 mM), 1:250 trypsin (50 μg/ml), and penicillin (100 units/ml) and neomycin (100 μg/ml). This medium will be referred to as the supplemented fetal calf serum medium. The other, which will be referred to as the calf serum medium, consists of MEM containing 15% calf serum.

Cell Synchronization

Populations of synchronous cells were obtained by growing cells as monolayer cultures in Bellco roller bottles (Bellco Glass, Inc., Vineland, N. J.) (280 × 110 mm). Loosely attached mitotic cells were shaken off the glass surface (4) and were collected by centrifugation. In several experiments, Colcemid (0.06 μg/ml) was added 1.5 hr before agitation to increase the size of the mitotic population (13). The addition of Colcemid and subsequent removal of the drug had no effect on the level of isotope incorporation or on the duration of G_1, which is defined in this paper as the time required for the population of cells to commence DNA synthesis. The medium containing the Colcemid was poured off and the mitotic cells were collected directly into serumless medium (12). The cells were gently centrifuged in a clinical centrifuge at 1500 rpm for 2 min, resuspended, and thoroughly dispersed in the appropriate medium. The mitotic index of the synchronous population was roughly 95% as determined by phase-contrast microscopy.

Isotopic Experiments

5-ml samples of the freshly suspended cells were transferred to a series of 25 cm^2 plastic T-flasks (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.) and were incubated at 37°C under an atmosphere of 95% air and 5% CO_2. In different experiments, the final cell number varied from 0.5 × 10^6 cells to 2 × 10^6 cells per flask. The rate of incorporation of labeled uridine into RNA was studied by exposing cells to uridine-5-^{3}H (1.5 μCi/ml, 8 Ci/mM) for 10-min periods at various times after collection of the mitotic cells. In parallel experiments, DNA synthesis was monitored by pulse labeling a separate series of duplicate flasks with thymidine-^{3}H (1.0 μCi/ml, 14.3 Ci/mmole). After the labeling period, the radioactive medium was quickly aspirated and cells were removed from the plastic surface with a Tris-buffered, 0.05% trypsin solution (14). The cells were transferred to a conical centrifuge tube and were sedimented at 4°C. The supernatant was removed and the pelleted cells were precipitated with 5% trichloroacetic acid (TCA) at 4°C. The precipitates were washed three times with ice-cold TCA, once with a 1:4 mixture of ethanol-ether, and once with anhydrous ether. The dried precipitates were then dissolved in 1 ml of 0.3 N KOH. In preliminary experiments, DNA and RNA were separated and radioactivity in each was determined. This was done by incubating samples in 1.0 ml of 0.3 N KOH for 18 hr at 37°C. This treatment hydrolyzes the RNA and allows precipitation of DNA upon addition of 0.12 ml of 50% TCA. More than 95% of the counts due to uridine-5-^{3}H was found in the KOH hydrolysate, while greater than 95% of the radioactivity due to

thymidine-^{3}H remained in the non-KOH hydrolyzable material. Hence, in subsequent experiments, cells were incubated separately with uridine-^{3}H or thymidine-^{3}H, and 0.1 ml of each 0.3 N KOH sample was introduced directly into counting vials without separating RNA from DNA. In experiments where the uptake of labeled uridine into the acid-soluble pool was determined, the labeled medium was aspirated, the cells were taken up in 8 ml of a buffered trypsin solution, and the samples were centrifuged to pellet cells. After the trypsin solution was removed, 1.0 ml of ice-cold 5% TCA was added to the cells, and the samples were allowed to stand in the cold for 15 min. The pellet was dispersed and the samples centrifuged. The supernatant was transferred to a separate tube and 0.1 ml was assayed for radioactivity. Label incorporated into RNA was determined from the remaining pellet. All samples were counted in 10 ml of scintillation fluid (0.2 g *P*-bis[2-(5-phenyloxazolyl)]-benzene (POPOP) and 5.0 g 2,5-diphenyloxazole (PPO) per liter toluene) and 0.2 ml Beckman solubilizer BBS 3 in a Packard Tri-Carb scintillation counter.

RESULTS

Rates of Uridine Incorporation into RNA during the Cell Cycle

CELLS CULTURED IN SUPPLEMENTED FETAL CALF SERUM MEDIUM: When this series of experiments was initiated, the cells had been continuously cultured in supplemented fetal calf serum medium. The rate of incorporation of uridine into RNA during the cell cycle of cells grown in this medium was examined; the results of one such experiment are illustrated in Fig. 1 *a*. Mitotic cells were collected and pulse labeled with either uridine-5-^{3}H or thymidine-^{3}H at intervals after synchronization. The solid triangles represent amounts of labeled uridine incorporated during 10-min periods at different times during the cell cycle, while the open circles reflect thymidine incorporation into DNA over the same period of time. As indicated by the data, the rate of uridine incorporation into RNA increases only slightly during G_1 and accelerates sharply at the time DNA synthesis commences.

The results of five such experiments are summarized in Table I *a*. For each experiment, the slopes of the uridine incorporation curve, calculated by the method of least squares, describes the change in rate of uridine incorporation during the period examined. The ratios of the slopes between the S phase and the G_1 phase are presented in the last column. The values in this column show that the rate of uridine incorporation increases two to 14 times more rapidly during the first part of the S period than during G_1.

CELLS CULTURED IN SUPPLEMENTED FETAL CALF SERUM MEDIUM AND TRANSFERRED TO CALF SERUM MEDIUM: When cells were grown in the supplemented fetal calf serum medium, the rate of uridine incorporation increased markedly at about the time DNA synthesis was initiated. To see whether this acceleration in uridine incorporation could be dissociated from the initiation of DNA synthesis, the cells were transferred for 24 hr to a less-supplemented medium (calf serum medium) containing only MEM and 15% calf serum which was expected to lengthen the duration of G_1. 24 hr after transfer to calf serum medium, mitotic cells were collected, seeded in T-flasks, and then pulse labeled with uridine-5-^{3}H at intervals during the cell cycle. The cells displayed the incorporation pattern described by the experiment in Fig. 1 *b*. The incorporation of thymidine-^{3}H into DNA, represented by open circles, indicates that the duration of G_1 under these growth conditions is the same as in the previous set of experiments. The pattern of uridine incorporation into RNA, however, is quite different. In this case, it increases linearly throughout G_1 and the early part of the DNA synthetic phase, and, unlike that for cells cultured in the supplemented fetal calf serum medium, it displays no change in the slope at the onset of DNA synthesis. Table I *b* summarizes the results of three such experiments in which the ratio of the slopes between the early S phase and G_1 approaches unity.

CELLS CULTURED IN CALF SERUM MEDIUM: To exclude the possibility that the induction of the continuous pattern of increase in uridine incorporation is not simply a transitory metabolic adjustment, the cells were cultured in calf serum medium for 2 months. After that period of time, the cells were synchronized by collection of mitotic cells, and were pulse labeled with uridine-5-^{3}H at intervals during the cell cycle. The experiment illustrated in Fig. 2 shows that, after the cells were cultured for this period of time in calf serum, there is little or no change in the duration of the G_1 or S phases, and that the linear pattern of uridine incorporation during G_1 and the first part of S is maintained. Four experiments performed with cells cultured in calf serum medium are described

TABLE I

Slopes Calculated from Curves Showing Changes in Rates of Uridine-5-^3H Incorporation into RNA during G_1 and Early S

Culture medium	Cell number per flask at beginning of experiment	Slope (G_1)	Slope (S)	Slope S/slope G_1
	a			
FCS*	0.9×10^6	12	178	14.8
	1.0×10^6	70	310	4.4
	1.3×10^6	130	243	1.8
	1.6×10^6	63	447	7.0
	2.0×10^6	34	247	7.2
Mean ± standard error				5.1 ± 3.35
	b			
FCS* → CS‡ (24 hr)	0.6×10^6	42	27	0.6
	1.2×10^6	270	260	0.9
	1.8×10^6	550	825	1.5
Mean ± standard error				1.0 ± 0.27
	c			
CS‡	1.4×10^6	102	71	0.6
	1.6×10^6	410	286	0.6
	2.0×10^6	1173	690	0.5
	2.0×10^6	2082	1571	0.7
Mean ± standard error				0.6 ± 0.13
	d			
FCS → CS (24 hr) → FCS (24 hr)	0.8×10^6	11	217	19.7
	e			
MEM + 15% fetal calf serum only	1.2×10^6	31	223	7.1
	f			
FCS (10 min pulse)	10^6	712	2455	3.4
FCS (30 min pulse)	10^6	2498	7246	2.9

Synchronized cells were pulse labeled with uridine-5-^3H at different times during the cell cycle after they had been cultured in (*a*) fetal calf serum medium, (*b*) fetal calf serum medium and transferred to calf serum medium for 24 hr, (*c*) calf serum medium, (*d*) fetal calf serum medium, transferred to calf serum medium for 24 hr, and returned to fetal calf serum medium for 24 hr, (*e*) MEM + 15% fetal calf serum only, and (*f*) fetal calf serum medium and labeled for 10 or 30 min. The slopes of all uridine-^3H incorporation curves during the G_1 and early S phases were calculated by the method of least squares.

* FCS refers to the supplemented fetal calf serum medium.

‡ CS refers to calf serum medium.

in Table I *c*. Consistently, the ratios of the slopes between S and G_1 approximate one.

Reversibility of the Patterns

To see whether the patterns of uridine incorporation rates are reversible, cells which had been cultured in supplemented fetal calf serum medium were transferred to calf serum medium for 24 hr. They were then returned to fresh supplemented fetal calf serum medium for an additional 24 hr. The cells were synchronized and pulse labeled at different times in the cell cycle as before, and the

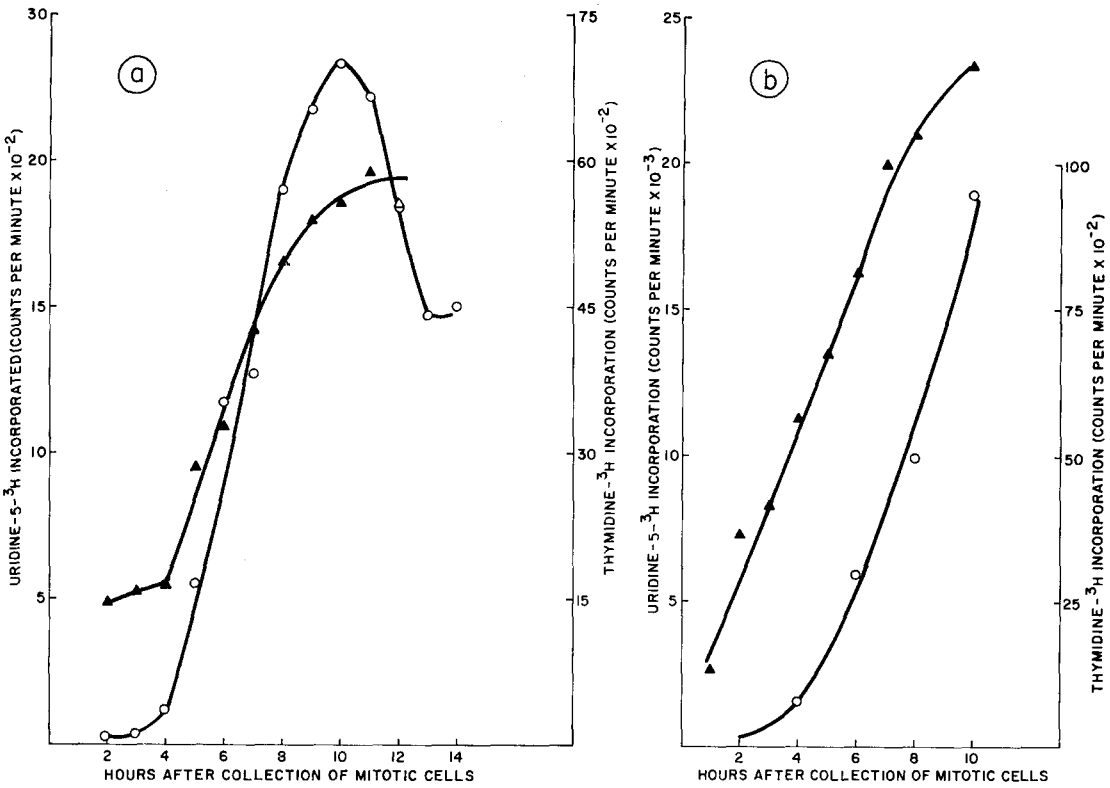

FIGURE 1 Cells cultured in (*a*) supplemented fetal calf serum medium or (*b*) supplemented fetal calf serum medium and then transferred to calf serum medium for 24 hr were synchronized by collection of mitotic cells. Equal portions of cells were labeled for 10 min with either thymidine-^{3}H or uridine-5-^{3}H at different times during the cell cycle. Triangles represent incorporation of uridine-5-^{3}H into RNA; circles represent incorporation of thymidine-^{3}H into DNA.

rates of uridine and thymidine uptake were determined. It had previously been shown (Fig. 1 *b* and Table I *b*) that 24 hr of culture in calf serum medium resulted in a linear increase in rate of uridine incorporation during G_1 and early S. When these same cells were returned to supplemented fetal calf serum medium, they reverted to their original pattern of incorporation as demonstrated by the calculated slopes presented in Table I *d*. This indicates that the cells can assume either pattern of uridine incorporation within 24 hr when cultured in the appropriate medium.

Medium Constituent Responsible for the Changes in Uridine Incorporation Patterns

Cells cultured in the supplemented fetal calf serum medium were transferred for 30 hr to MEM containing only 15% calf serum with none of the other additives normally present in the supplemented fetal calf serum medium. Fig. 1 *b* and Table I *b* show that within 24 hr of culture in calf serum medium, the cells exhibit a linear increase in their rate of uridine incorporation. The calculated slopes shown in Table I *e* demonstrate that in a medium containing 15% fetal calf serum but lacking the lactalbumin hydrolysate, glutamine, and trypsin, these cells, in contrast to cells transferred to calf serum medium, continue to display the sharp acceleration in rate of uridine incorporation as DNA synthesis commences. Under the experimental conditions used, the presence of 15% fetal calf serum alone in the medium, therefore, appears sufficient to maintain the pattern of uridine incorporation into RNA exhibited by cells cultured in the supplemented fetal calf serum medium.

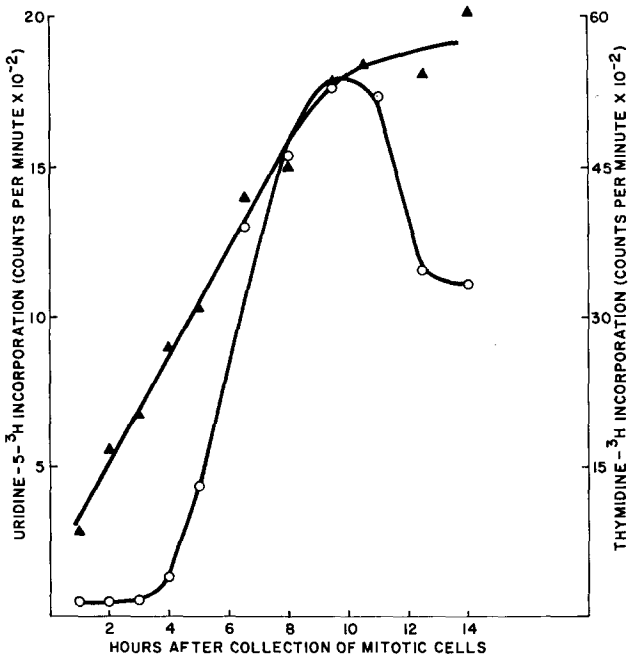

FIGURE 2 Cells cultured in calf serum medium were synchronized by collection of mitotic cells. Equal portions of cells were labeled for 10 min with either thymidine-^{3}H or uridine-5-^{3}H at different times during the cell cycle. Triangles represent incorporation of uridine-5-^{3}H into RNA; circles represent incorporation of thymidine-^{3}H into DNA.

The Pattern of Uridine-^{3}H Incorporation after a 10 or 30 Min Labeling Period

The possibility that the 10 min pulse time was too short to allow equilibration of the labeled uridine with the endogenous uridine nucleotide pool was examined. The absence of such equilibration could contribute to the patterns observed. Cells cultured in fetal calf serum medium were synchronized and were pulse labeled during the cell cycle with uridine-5-^{3}H for either 10 or 30 min. For both labeling periods, the patterns were nonlinear (Table I *f*).

Levels and Patterns of Uridine Incorporation into RNA and Uptake of Uridine into the Acid-Soluble Pool

Comparisons were made of the uptake of uridine into the acid-soluble pool and of the levels of uridine incorporation into RNA between cells that had been continuously cultured in fetal calf serum medium and cells from parallel cultures that had been transferred for 22 hr to calf serum medium. Examination of Fig. 3 *a* and 3 *b* reveals that on a per cell basis both the rate of uridine incorporation into RNA and the level of labeled uridine in the acid-soluble pool are about three times greater in cells transferred to calf serum medium than in those maintained on supplemented fetal calf serum medium. In both cases, the rate of uridine-^{3}H incorporation into RNA increases six- to sevenfold over 8 hr, and is closely paralleled by the rate of uptake of uridine-^{3}H into the acid-soluble pool. The *pattern* of increase in the rate of uridine-^{3}H incorporation into RNA is also similar to the pattern of increase of uridine-^{3}H uptake into the acid-soluble pool in the respective calf serum and fetal calf serum media (Figs. 3 *a*, 3 *b*). These results suggest that under the experimental conditions used, the rate of uridine incorporation into RNA is not a measure of the rate of RNA synthesis but rather a measure of the rate of entry of uridine into the cell.

Effect of Exogenous Uridine Concentrations upon the Patterns of Uridine Uptake and Incorporation

The similarities between the patterns of increase in rate of uridine incorporation into RNA and

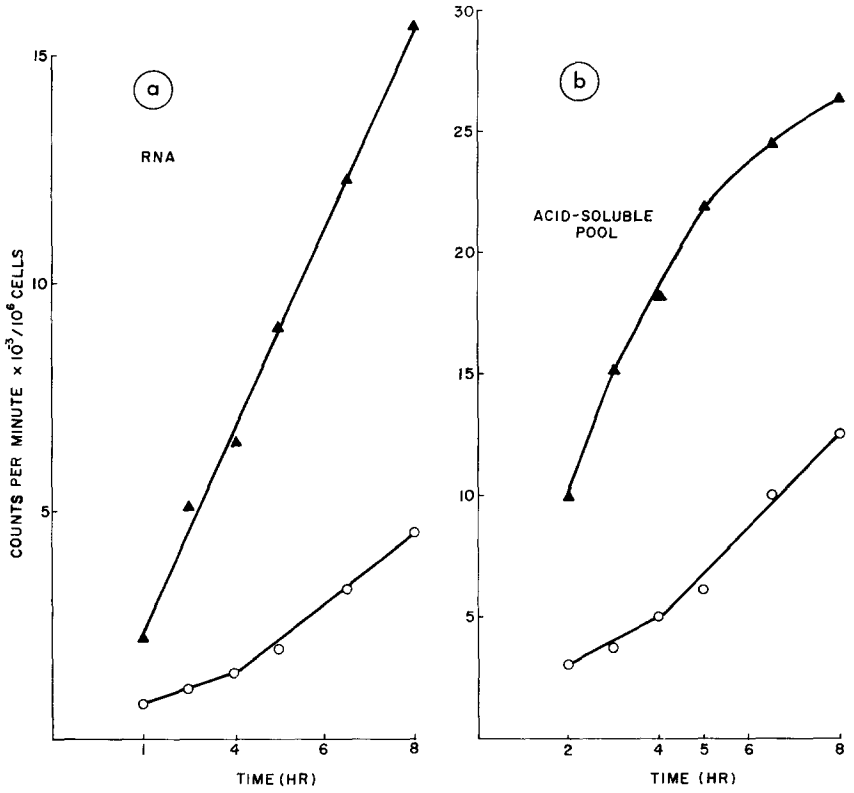

FIGURE 3 Incorporation of uridine-5-^{3}H into RNA (*a*) and uptake of uridine-5-^{3}H into the acid-soluble pool (*b*) were compared for cells growing on calf serum medium (triangles) and supplemented fetal calf serum medium (circles). Cells which had been grown on supplemented fetal calf serum medium were subcultured into six roller bottles. 24 hr before mitotic cells were collected, the medium in three of the bottles was replaced with calf serum medium while the medium in the other three bottles was replaced with fresh supplemented fetal calf serum medium. Mitotic cells were collected, suspended in the appropriate medium, and portions of each were labeled for 10 min at different times up to 8 hr.

rate of uridine uptake into the acid-soluble pool suggested that a transport mechanism for uridine might be operative. In an attempt to overcome any such transport system, experiments were carried out using very high exogenous uridine concentrations so that the primary mode of uridine entry into the cell would be by diffusion rather than transport. Synchronized cells in supplemented fetal calf serum medium were divided into two series of samples. One series was pulse labeled at different times during the cell cycle with 2 μCi/ml of uridine-5-^{3}H at a final concentration of 0.25 μM uridine. The parallel series was treated simultaneously but with a final uridine concentration of 200 μM. The results of one such experiment are presented in Fig. 4. Cells pulse labeled with 0.25 μM uridine displayed the expected nonlinear pattern of increase in the rate of uridine-^{3}H incorporation into RNA (Fig. 4 *a*) and of uridine-^{3}H uptake into the acid-soluble pool (Fig. 4 *b*). In contrast to the control population, cells pulse labeled with 200 μM uridine exhibited a linear pattern of increase in the rate of uridine-^{3}H incorporation into RNA (Fig. 4 *a*) and of uridine-^{3}H uptake into the acid-soluble pool (Fig. 4 *b*). The induction of a linear pattern of both uridine incorporation into RNA and uptake into the acid-soluble pool with 200 μM uridine is consistent with the presence of a uridine transport system such that at low exogenous uridine concentrations the rate of entry of uridine into the cell limits the rate of uridine incorporation into RNA.

DISCUSSION

Two apparent patterns of increase in RNA synthetic rate during the cell cycle have been described

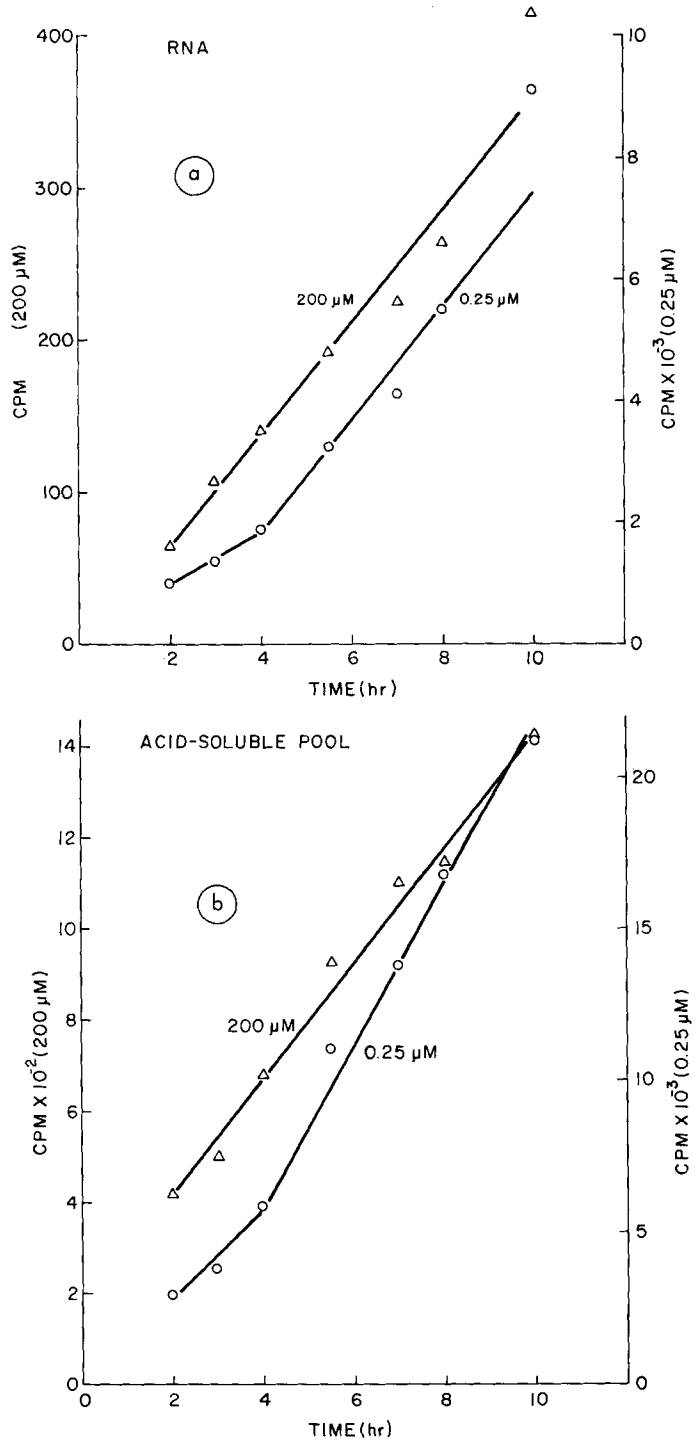

FIGURE 4 Incorporation of uridine-5-^{3}H into RNA (*a*) and uptake of uridine-5-^{3}H into the acid-soluble pool (*b*). Cells were cultured in supplemented fetal calf serum and synchronized by collection of mitotic cells. Equal portions of cells were labeled for 10 min at different times during the cycle with 2 μCi/ml of uridine-5-^{3}H at a final uridine concentration of either 0.25 μ/M (triangles) or 200 μM (circles).

in the literature. Several authors (7, 8, 9), using radioactive precursors of RNA, have reported a linear increase in synthetic rate during G_1 and S, whereas others (4, 5, 6) have claimed a relatively constant rate of synthesis during G_1 with a marked acceleration at about the time DNA synthesis is initiated. Suggested sources for the differences in RNA synthetic patterns have included differences in cell type (5), the duration of interphase (6), the neoplastic or normal origin of the cells (15), or volume differences in the cells examined (16). The present study demonstrates that when uridine-5-^{3}H is used as a precursor of RNA, patterns of incorporation resembling both reported patterns of increase in synthesis can be induced without apparent changes in the cell cycle. Under the experimental conditions used, the pattern of increase in rate of uridine uptake into the acid-soluble pool is similar to the observed pattern of incorporation into RNA.

The transition from the biphasic pattern to the linear pattern of uridine incorporation or uptake can be induced within 24 hr by transferring cells growing in the supplemented fetal calf serum medium to the calf serum medium. These same cells, when transferred back to supplemented fetal calf serum medium, revert to their original nonlinear pattern. To exclude the possibility that observed changes in the kinetics of uridine incorporation resulted from a transiently perturbed metabolism of cells adapting to a new environment, cells were grown on calf serum medium for a minimum of 2 months. These cells retained the linearly increasing rate of incorporation that they had acquired within the first 24 hr of growth on the calf serum medium. When cells which had been cultured on supplemented fetal calf serum medium were transferred to MEM containing only 15% fetal calf serum for 30 hr, the cells still retained their nonlinear pattern of increase in RNA synthetic rate. These results suggest that under the culture conditions used the fetal calf serum may play some role in maintaining the nonlinear pattern of uridine uptake and incorporation rates during the cell cycle.

Although serum content appears to play a critical role in determining the pattern of uridine uptake and incorporation in these experiments, there seems to be no such correlation for the patterns reported in the literature. Pfeiffer and Tolmach (6) (10% calf serum and 5% fetal calf serum) observed a nonlinear pattern in HeLa S3 cells, whereas Scharff and Robbins (7) (3.5% calf serum and 3.5% fetal calf serum) observed a linear pattern also using HeLa S3 cells. Klevecz and Stubblefield (5) (20% fetal calf serum) found a nonlinear pattern of increase in the Chinese hamster Don line, while Enger and Tobey (9) (10% calf serum and 5% fetal calf serum) found a linear mode of increase in a Chinese hamster ovarian line. Seed (10) observed a nonlinear increase in chromatin (nuclear) RNA synthetic rate in normal monkey kidney cells and a linear increase in HeLa cells, using the same medium (20% human serum) in both cases. That the pattern of uridine incorporation is not altered by growing cells as monolayers rather than in suspension culture was shown by Enger and Tobey (9) who found similar patterns (linear) in each case.

The possibility that differences in cell density in the separate experiments influenced the pattern of increase of uridine uptake and incorporation was ruled out. The cell concentration between experiments varied from 0.6×10^6 cells per flask to 2×10^6 cells per flask, but even at the higher concentration the cells were not confluent. There is considerable overlap in cell concentration between experiments in which calf serum medium was used and those in which supplemented fetal calf serum medium was used, but in each case the pattern of increase in uridine uptake and incorporation correlated with the type of serum used and not with cell density (Table I).

The results reported in the literature are primarily data from isotope incorporation experiments and must be interpreted with caution. Changes in rates of uridine incorporation may not be entirely due to changes in rates of RNA synthesis but might also be attributed to changes in the specific activity of the uridine triphosphate (UTP) precursor pool. An increase in the permeability of the cells to exogenous uridine-^{3}H or a natural decrease in the endogenous UTP pool size during the cell cycle could result in a higher specific activity of the internal UTP pool when uridine-^{3}H is added to the medium. That a marked increase in the uptake of uridine-^{3}H into the acid-soluble pool as well as in incorporation into RNA occurs during the cell cycle is consistent with this possibility. Misinterpretation of such incorporation studies has been discussed by Plagemann (17) who found that addition of phenethyl alcohol to cultured cells resulted in a decrease of labeled uridine incorpora-

tion into RNA, not because of inhibition of RNA synthesis but because the permeability to uridine had been significantly reduced. Similar observations have been reported by Cunningham and Pardee (26) and Hersko et al. (27) upon adding or removing serum from cultured cells and by Ceccarini and Eagle (28) by adjusting the pH of the culture medium.

Aside from changes in cell permeability and endogenous UTP pool sizes, disparities between uridine-^{3}H incorporation data and true rates of RNA synthesis could arise from variations in uridine kinase activity. Naturally increasing levels of uridine kinase activity during the cell cycle or the induction of uridine kinase during the cell cycle would likely result in elevated specific activities of the UTP pool with concomitant increases in rates of uridine-^{3}H incorporation. Hausen et al. (18) have demonstrated that uridine kinase is induced in lymphocytes stimulated with phytohemagglutinin and that the increased level of uridine incorporation reflects an elevated uridine kinase activity as well as an increased rate of RNA synthesis. To explore the relationship between our uridine incorporation data and the relative rates of RNA synthesis, we are currently examining how ribonucleoside triphosphate pool sizes and specific activities behave during the cell cycle and whether changes in uridine kinase activities occur under conditions used in these experiments.

Circumventing the use of radioactive precursors, Seed has examined the rate of accumulation of RNA by using a combination of microcinematography and microspectrophotometry and found that the total amount of RNA per cell doubles over the cell cycle (19, 20, 21, 22). In view of the existence of large amounts of rapidly turning-over nuclear RNA (23), one cannot interpret Seed's data as a measure of rate of RNA synthesis, but rather as a measure of net increase in RNA content.

In this strain of Chinese hamster V79 cells, and under the culture conditions employed, the rates of uridine incorporation into RNA increase six- to 10-fold over the cell cycle in either calf serum medium or fetal calf serum medium. On a per cell basis, cells transferred to calf serum medium incorporate about three times as much uridine into RNA as cells cultured on fetal calf serum medium (Fig. 3 *a*). Likewise, the amount of labeled uridine that enters the acid-soluble pool during a 10 min pulse is about three times greater in cells that had been transferred to calf serum medium (Fig. 3 *b*). Changes in the pattern of increase in the rate of uridine uptake into the acid-soluble pool closely follow the changes in the pattern of increase in the rate of uridine incorporation into RNA (Figs. 3 *a*, 3 *b*). This suggests that, under the conditions used, the rates of uridine incorporation into RNA may reflect the rates of transport of uridine into the cell rather than the rate of RNA synthesis. Consistent with this observation is the finding that when cells cultured in supplemented fetal calf serum medium are incubated with very high uridine concentrations, they display a linear rather than nonlinear pattern of increase in the rate of uridine incorporation and uptake. The high uridine concentrations should saturate the transport system so that the primary mode of uridine entry into the cell is by diffusion rather than by any transport mechanism. Pfeiffer and Tolmach (6) reported the same nonlinear pattern with 0.2 μM uridine and 20 μM uridine, but it is possible that they did not use a sufficiently high uridine concentration to effect a change in pattern. Under the conditions used in the present experiments, the cells continued to display a nonlinear pattern with uridine concentrations as high as 100 μM (unpublished results).

The rate of uridine uptake and incorporation is enhanced for cells in medium containing calf serum. One could postulate an inducer in calf serum which stimulates the rate of uridine transport and, therefore, the rate of uridine incorporation. An alternate argument can be made, however, for an inhibitor of the uridine transport mechanism in fetal calf serum which produces the diminished rates of uridine uptake and incorporation. If cells in G_1 were more sensitive to the inhibitor than cells later in the cell cycle, one would predict the nonlinear increase in rate of uptake and incorporation that is displayed by cells in supplemented fetal calf serum. The fact that high exogenous uridine concentrations (200 μM), which favor uridine entry by diffusion rather than by transport, induce a linear pattern of increase in rate of uridine uptake and incorporation support the notion that an inhibitor of uridine transport exists in fetal calf serum and that cells in G_1 are differentially sensitive to this inhibitor.

That changes in uridine transport are responsible for the two patterns of uridine incorporation is supported by the report of Plagemann and Roth (24) who noted that transport of uridine into Novikoff hepatoma cells is the rate-limiting step

in the incorporation of uridine into RNA. Peters and Hausen (25), using stimulated lymphocytes, have come to the same conclusion. Several investigators have reported transport systems for several different nucleic acid precursors ([1], 26, 27), and two of these reports have demonstrated that the transport of adenosine and uridine is differentially affected upon changes in serum content (26, 27). Changes in transport efficiency during the cell cycle and in the presence of different sera are currently being investigated.

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REFERENCES

1. HODGE, L. D., T. W. BORUN, E. ROBBINS, and M. D. SCHARFF. 1969. Studies on the regulation of DNA and protein synthesis in synchronized HeLa cells. *In* Biochemistry of Cell Division. R. Baserga, editor. Charles C Thomas, Publisher, Springfield, Illinois. 15–37.
2. ZYLBER, E. A., and S. PENMAN. 1971. Synthesis of 5S and 4S RNA in metaphase-arrested HeLa cells. *Science (Washington).* **172:**947.
3. KLEVECZ, R. R. 1969. Temporal order in mammalian cells. I. The periodic synthesis of lactate dehydrogenase in the cell cycle. *J. Cell Biol.* **43:**207.
4. TERASIMA, T., and L. J. TOLMACH. 1963. Growth and nucleic acid synthesis in synchronously dividing populations of HeLa cells. *Exp. Cell Res.* **30:**344.
5. KLEVECZ, R. R., and E. STUBBLEFIELD. 1967. RNA synthesis in relation to DNA replication in synchronized Chinese hamster cell cultures. *J. Exp. Zool.* **165:**259.
6. PFEIFFER, S. E., and L. J. TOLMACH. 1968. RNA synthesis in synchronously growing populations of HeLa S3 cells. I. Rate of total RNA synthesis and its relationship to DNA synthesis. *J. Cell. Physiol.* **71:**77.
7. SCHARFF, M. D., and E. ROBBINS. 1965. Synthesis of ribosomal RNA in synchronized HeLa cells. *Nature (London).* **208:**464.
8. KIM, J. H., and A. G. PEREZ. 1965. Ribonucleic acid synthesis in synchronously dividing populations of HeLa cells. *Nature (London).* **207:**974.
9. ENGER, M. D., and R. A. TOBEY. 1969. RNA synthesis in Chinese hamster cells. II. Increase in rate of RNA synthesis during G_1. *J. Cell Biol.* **42:**308.
10. SEED, J. 1963. Studies of biochemistry and physiology of normal and tumor strain cells. *Nature (London).* **198:**147.
11. FORD, D. K., and G. YERGANIAN. 1958. Observations on the chromosomes of Chinese hamster cells in tissue culture. *J. Nat. Cancer Inst.* **21:**393.
12. EAGLE, H. 1959. Amino acid metabolism in mammalian cell culture. *Science (Washington).* **130:**432.
13. STUBBLEFIELD, E., and R. KLEVECZ. 1965. Synchronization of Chinese hamster cells by reversal of colcemid inhibition. *Exp. Cell Res.* **40:** 660.
14. MADIN, S. H., and N. B. DARBY. 1958. Established kidney cell lines of normal adult bovine and ovine origin. *Proc. Soc. Exp. Biol. Med.* **98:** 574.
15. SEED, J. 1963. Growth control mechanisms in normal and tumor strain cells. *Nature (London).* **198:**153.
16. SINCLAIR, W. K., and D. ROSS. 1969. Modes of growth in mammalian cells. *Biophys. J.* **9:**1056.
17. PLAGEMANN, P. G. W. 1970. Effects of phenethyl alcohol on transport reactions, nucleotide pools and macromolecular synthesis in Novikoff rat hepatoma cells growing in suspension culture. *J. Cell. Physiol.* **75:** 315.
18. HAUSEN, P., and H. STEIN. 1968. On the synthesis of RNA in lymphocytes stimulated by phytohemagglutinin. I. Induction of uridine-kinase and the conversion of uridine to UTP. *Eur. J. Biochem.* **4:**401.
19. SEED, J. 1966. The synthesis of DNA, RNA and nuclear protein in normal and tumor strain cells. I. Fresh embryo human cells. *J. Cell Biol.* **28:**233.
20. SEED, J. 1966. The synthesis of DNA, RNA and nuclear protein in normal and tumor strain cells. II. Fresh embryo mouse cells. *J. Cell Biol.* **28:**249.
21. SEED, J. 1966. The synthesis of DNA, RNA and nuclear protein in normal and tumor strain cells. III. Mouse ascites tumor cells. *J. Cell Biol.* **28:**257.
22. SEED, J. 1966. The synthesis of DNA, RNA and nuclear protein in normal and tumor strain cells. IV. HeLa tumor strain cells. *J. Cell Biol.* **28:**263.

[1] Stambrook, P. J., and J. E. Sisken. Manuscript in preparation.

23. SHEARER, R. W., and B. J. MCCARTHY. 1967. Evidence for ribonucleic acid molecules restricted to the cell nucleus. *Biochemistry*. **6**:283.
24. PLAGEMANN, P. G. W., and M. F. ROTH. 1969. Permeation as the rate-limiting step in the phosphorylation of uridine and choline and their incorporation into macromolecules by Novikoff hepatoma cells. Inhibition by phenethyl alcohol, persantin and adenosine. *Biochemistry*. **12**:4782.
25. PETERS, J. H., and P. HAUSEN. 1971. Effect of phytohemagglutinin on lymphocyte membrane transport. I. Stimulation of uridine uptake. *Eur. J. Biochem.* **19**:502.
26. CUNNINGHAM, D. D., and A. B. PARDEE. 1969. Transport changes rapidly initiated by serum addition to "contact inhibited" 3T3 cells. *Proc. Nat. Acad. Sci. U. S. A.* **64**:1049.
27. HERSHKO, A., P. MAMONT, R. SCHIELDS, and G. M. TOMPKINS. 1971. "Pleiotypic response." *Nature (London)*. **232**:206.
28. CECCARINI, C., and H. EAGLE. 1971. pH as a determinant of cellular growth and contact inhibition. *Proc. Nat. Acad. Sci. U. S. A.* **68**:229.