PROTEIN SYNTHESIS AND RNA SYNTHESIS DURING MITOSIS IN ANIMAL CELLS

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ABSTRACT

Protein synthesis and RNA synthesis during mitosis were studied by autoradiography on mammalian tissue culture cells. Protein synthesis was followed by incubating hamster epithelial and human amnion cells for 10 or 15 minutes with phenylalanine-C^{14}. To study RNA synthesis the hamster cells were incubated for 10 minutes with uridine-C^{14}. Comparisons of the synthetic capacity of the interphase and mitotic cells were then made using whole cell grain counts. The rate of RNA synthesis decreased during prophase and reached a low of 13 to 16 per cent of the average interphase rate during metaphase-anaphase. Protein synthesis in the hamster cells showed a 42 per cent increase during prophase with a subsequent return to the average interphase value during metaphase-anaphase. The human amnion cells showed no significant change at prophase but there was a 52 to 56 per cent drop in phenylalanine incorporation at metaphase-anaphase as compared to the average interphase rate. Colcemide was used on the hamster cells to study the effect of a prolonged mitotic condition on protein and RNA synthesis. Under this condition, uridine incorporation was extremely low whereas phenylalanine incorporation was still relatively high. The drastic reduction of RNA synthesis observed under mitotic conditions is believed to be due to the coiled condition of the chromosomes. The lack of a comparable reduction in protein synthesis during mitosis is interpreted as evidence for the presence in these cells of a relatively stable messenger RNA.

INTRODUCTION

Studies of the growth pattern during the life cycle of various protista have yielded conflicting evidence on the metabolic status of the dividing cell. Some investigators find no evidence of disruption in the general interphase growth pattern during the time of cell division (Adolph and Bayne-Jones, 1932; Knaysi, 1940; Mitchison, 1957, 1958; Abbo and Pardee, 1960; Williamson and Scopes, 1961). Other data, however, show marked reduction in growth rate during division (Bayne-Jones and Adolph, 1932; Knaysi, 1940; Zeuthen, 1953; Lindegren and Haddad, 1954; Prescott, 1955; Maruyama, 1956; Christensson, 1959; Hamburger and Zeuthen, 1960; Satir and Zeuthen, 1961) and, in some cases, there is evidence for a loss of mass at the time of division (Kimball, Caspersson, Svensson, and Carlson, 1959; Sandritter, Schiemer, Kraus, and Dörrien, 1960; Woodard, Gelber, and Swift, 1961).

Changes in cell morphology that occur during mitosis are so drastic that on this basis alone it might be predicted that there would be a change from the interphase pattern of metabolism. The chromosomes become condensed; the nuclear membrane breaks down; the nucleolus disappears; and the spindle and its related structures form, oc-

cupying a large fraction of the cell and thus disorganizing the interphase cytoplasmic structure. Fine structure cytology reveals that the endoplasmic reticulum is disorganized (Porter, 1954; Porter and Machado, 1960) and mitochondria are often pushed to the periphery (Porter, 1954; Porter and Machado, 1960; Gross, 1957; Kurosumi, 1958).

Uptake of $P^{32}O_4$ and $S^{35}O_4$ has been studied in several cell types and found to decline during division (Zeuthen, 1951; Taylor and Taylor, 1953; Mazia and Prescott, 1954; Taylor, 1958; Hamburger and Zeuthen, 1960; de Terra, 1960). Much work has been done recently on the uptake of specific precursors of protein, DNA, and RNA during the life cycle of the cell (Siskin, 1959; Feinendegen, Bond, Shreeve, and Painter, 1960; Taylor, 1959, 1960; Woodard, Rasch, and Swift, 1961; Baserga, 1962 *a*, 1962 *b*; Mitchison and Wilbur, 1962; Prescott and Bender, 1962). In general, mitosis is found to be a period of depressed metabolic activity.

Except for the few instances when DNA synthesis extends into prophase or begins in telophase, most cells synthesize DNA well before division (Taylor, 1957). The work of Taylor, Woods, and Hughes (1957) with colchicine, and of Bibring (cited by Bucher and Mazia, 1960) with mercaptoethanol-treated cells supports the conclusion that condensed chromosomes do not synthesize DNA.

Taylor (1960) and Prescott and Bender (1962) report that RNA synthesis completely stops when the chromosomes condense in mitosis. Das (1963) finds that although condensed chromosomes no longer synthesize RNA, it is only after the nucleolus breaks down that RNA synthesis comes to a complete halt. Prescott and Bender, however, state that RNA synthesis completely stops in prophase shortly before the nucleoli disappear.

The following experiments were undertaken to study the metabolic status of some mammalian tissue culture cells during the course of mitosis in regard to protein and RNA synthesis. Rapidly growing hamster and human amnion cells were pulse labeled with phenylalanine-C^{14} or uridine-C^{14} and the incorporation was quantitatively evaluated by autoradiography. Comparisons of this work and similar studies carried out by other investigators are made in the discussion.

MATERIALS AND METHODS

Two mammalian tissue culture lines were used in the following experiments. Most of the work was done on hamster epithelial cells isolated in 1958 in the laboratory of Dr. George Yerganian and carried since 1960 by the Naval Biological Laboratory, School of Public Health, University of California, Berkeley, California. The other cells were human amnion, isolated at the Virus Laboratory, University of California, Berkeley, California (Zitcer and Dunnebacke, 1957).

Culture medium for the hamster cells was composed of 80 per cent medium NCTC 109 (McQuilken, Evans, and Earle, 1957), 10 per cent fetal bovine serum and 10 per cent penicillin (100 units/ml)-streptomycin (50 μg/ml) solution; the human amnion cells were grown in a medium composed of 10 per cent of a 10 × concentrate of medium 199 (Morgan, Morton, and Parker, 1950) made up in 1 × Hanks' balanced salt solution, 5 per cent lamb serum, 75 per cent Hanks' balanced salt solution, and 10 per cent of the penicillin-streptomycin solution. All stock cultures were grown in 100-ml stoppered glass medicine bottles and kept at 37°C.

Cells to be used for the experiments were trypsinized (0.1 per cent trypsin-Versene solution in Puck's saline) from rapidly growing stock cultures and harvested by centrifugation. Approximately 3×10^4 cells in 0.2 ml of medium were pipetted onto 22-mm^2 glass coverslips and placed in individual 35 × 10-mm covered plastic Petri dishes. This volume of cells was allowed to remain on the coverslip for at least 4 hours to allow attachment of the cells to the coverslip before adding more medium to the dish to a final volume of 2 ml. The coverslip cultures were incubated in a 5 per cent CO_2 atmosphere at 37°C in a constant high humidity incubator.

Coverslip cultures used in experiments were allowed to grow until they were in the period of exponential growth. Insofar as possible, the growth conditions were constant for all cultures. Checks made on generation time, mitotic time, chromosome number, and appearance revealed no significant change during the time these experiments were carried on.

To study protein synthesis, the cells were fed DL-phenylalanine-C^{14} (California Corp. for Biochemical Research, Los Angeles, 4 to 12 mC/mM); uridine-C^{14} (New England Nuclear Corp., Boston, 12 to 16 mC/mM) was used to study RNA synthesis. Both precursors were added directly to the medium in which the cells had already been growing; phenylalanine to a final concentration of 20 μC/ml and uridine to 10 μC/ml. In some experiments colcemide (5×10^{-6} per cent) was used to study the effect of a

prolonged metaphase condition on the uptake of phenylalanine and uridine. It, too, was made up in medium in which the cells had previously been growing.

Generation time and length of time in the various stages of mitosis were determined for both cell lines (Edwards, Koch, Youcis, Freese, Laite, and Donalson, 1960). The length of time in the labeled precursor was adjusted to a time no greater than the time in any one mitotic stage: 10 minutes for the hamster cells and 15 minutes for the human amnion cells. Thus the cells in a given phase, when fixed, were in either that phase and/or the preceding phase during labeling.

Immediately after the cells were exposed to the labeled precursor, the medium was removed and the coverslips washed in unlabeled medium and then in several changes of Hanks' saline and fixed in methanol for at least 15 minutes. The coverslips were then attached to microscope slides with Euparal, cell side up, and allowed to dry for 3 days.

Slides labeled with uridine-C^{14} were then treated with deoxyribonuclease (Worthington Biochemical Corp.) for 3 hours at 37°C, at a concentration of 0.2 mg/ml, made up in 0.003 M $MgSO_4$ and adjusted to pH 6.7 with dilute NaOH. Enzyme-treated slides were rinsed in H_2O and again dried before further handling.

Kodak AR-10 stripping film was then applied to the slides (Doniach and Pelc, 1950) and the slides were exposed for 1 to 3 weeks. The resulting autoradiograms were stained with either toluidine blue (0.05 per cent toluidine blue in Walpole's buffer pH 4) or with Azure Eosin Giemsa (procedure of T. McClure, University of California Medical Center, San Francisco, California). After rinsing, the slides were air dried.

All grain counts were done visually at a magnification of 1000. Grain counts were made over single cells and corrected for background by determining the area of the cell with the aid of an ocular grid and counting grains in a cell-free area of known size. All counts over cells of experimental slides were significantly different from background. A minimum of 20 cells in each mitotic phase was counted on each slide. Checks made on previously counted cells gave an error of no more than 5 per cent.

Tritium-labeled precursors were used in earlier experiments but carbon-14 was substituted in the experiments described here because of the possibility of self-absorption in the more rounded and, therefore, presumably thicker, dividing cells. Although carbon-14 has a much longer range and, therefore, overcomes to a large extent the error due to possible differences in self-absorption among the cells, there is another geometric problem characteristic of long range emitters: one should expect differences in losses of grains in the counts among cells of varying geometry due to emissions hitting the emulsion outside the cell area. Therefore, controls were used in which the cells were labeled under the same conditions as were the experimentals but returned to unlabeled medium for some length of time greater than the division time (but less than the generation time) before being fixed. In this way, observations could be made on cells which were in mitosis at the time of fixation but which were labeled while they were in interphase.

However, for counts of control interphase cells under these conditions, correction must be made for the fact that during the time between labeling and fixation some cells had gone through mitosis, and, therefore, interphase counts include a group of daughter cells with half the amount of label of the parent cell. A formula for this correction may readily be derived by use of the formula for cumulative phase index given by Edwards *et al.* (1960, formula 1). Let x be the corrected mean interphase grain count. Then the observed mean interphase grain count is equal to:

$$\frac{p_1}{p_2} \cdot \frac{x}{2} + \frac{p_2 - p_1}{p_2} \cdot x$$

where p_2 is the fraction of cells in interphase and p_1 is the fraction of cells which have divided between the time of labeling and the time of fixation. Referring to formula 1 of Edwards *et al.*, we see that:

$$p_1 = 2(1 - 2^{-t_1/T})$$

$$p_2 = 2(1 - 2^{-t_2/T})$$

Where T = generation time (15 hours in the hamster cells and 30 hours in the human amnion cells); t_1 = time elapsed after labeling (time in unlabeled medium before fixation; note that t_1 is always less than t_2 in this experiment); t_2 = time in interphase (generation time minus mitotic time).

Thus, the corrected mean interphase grain count can be calculated if the generation time, the time elapsed between labeling and fixation, and the time in interphase are known. No correction was made for the small per cent of cells in mitosis when labeled and in interphase when fixed.

RESULTS

Uridine Incorporation

HAMSTER CELLS: A drastic reduction in the incorporation of uridine occurred during mitosis as seen from the data in Table I and Fig. 1. As

TABLE I

Results of Whole Cell Grain Counts over Hamster Cells Exposed to Uridine-C^{14} for 10 Minutes

Experiment	Interphase	Prophase	Metaphase	Anaphase	¶Telophase
Slide 1	*33.6 ± 2.1	18.4 ± 2.1	3.4 ± 0.8	3.1 ± 0.5	9.5 ± 1.8
	‡247.1 ± 15.4	135.3 ± 15.4	25.0 ± 5.9	22.8 ± 3.7	69.8 ± 13.2
Slide 2	38.8 ± 4.1	21.3 ± 2.0	5.2 ± 1.0	4.6 ± 0.6	10.7 ± 1.4
	240.7 ± 25.4	132.1 ± 12.4	32.2 ± 6.2	28.5 ± 3.7	66.4 ± 8.7
Slide 3	24.6 ± 2.1	9.4 ± 1.2	1.2 ± 0.2	2.2 ± 0.6	6.8 ± 0.9
	278.3 ± 23.8	106.3 ± 13.6	13.6 ± 2.3	24.9 ± 6.8	76.9 ± 10.2
§Average	255.4 ± 12.7	124.6 ± 8.0	23.6 ± 3.0	25.4 ± 2.9	71.0 ± 6.3
‖Corrected average	100.0 ± 6.6	56.5 ± 4.3	12.6 ± 1.7	16.4 ± 2.1	37.6 ± 3.8

* Average grain counts with standard error of the mean based on 20 cells per phase.

‡ Normalized average grain counts obtained by dividing the average grain count per phase by the mean grain count of that slide. This procedure overcomes differences among replicate slides due to differences in time of film exposure and allows them to be averaged.

§ Average of the normalized average grain counts of replicate experiments.

‖ Normalized average grain count of each mitotic phase corrected for self-absorption, etc., due to the geometry of cells in that phase, by dividing by the normalized average grain count for control cells in the same phase. The resulting interphase value was then set equal to 100 to facilitate comparison. These values from Tables I, II, and III are plotted in Fig. 1.

¶ Telophase counts are the sum of counts over sister cells.

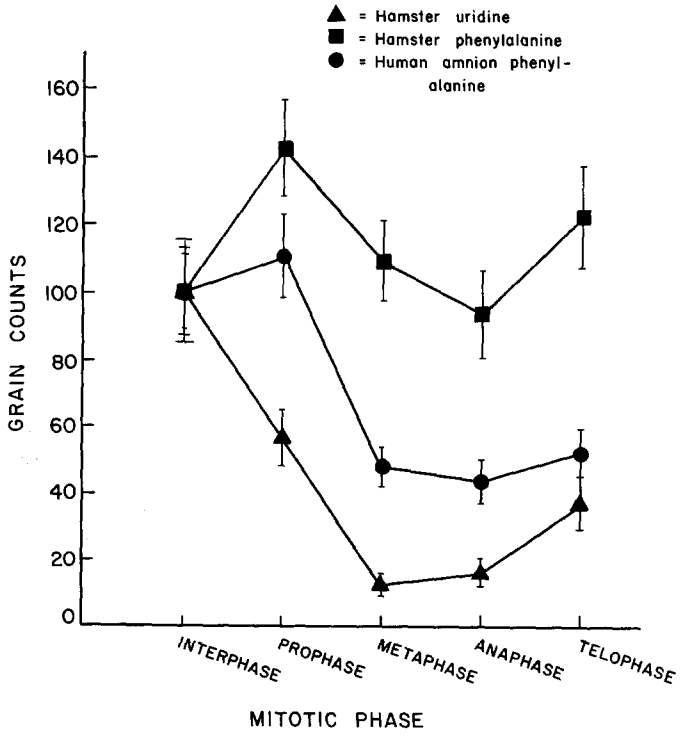

FIGURE 1 Normalized average grain counts corrected for self-absorption, etc. 95 per cent confidence limits are included for every point.

TABLE II

Results of Whole Cell Grain Counts over Hamster Cells Exposed to Phenylalanine-C^{14} for 10 Minutes

Experiment		Interphase	Prophase	Metaphase	Anaphase	¶Telophase
Slide 1	*	22.4 ± 1.1	32.0 ± 1.2	20.0 ± 1.2	10.6 ± 1.2	22.7 ± 1.8
	‡	104.0 ± 5.1	148.6 ± 5.6	92.8 ± 5.6	49.2 ± 5.6	105.4 ± 8.4
Slide 2		55.2 ± 4.0	67.9 ± 2.8	45.5 ± 2.6	34.6 ± 2.3	51.3 ± 3.6
		108.5 ± 7.9	133.4 ± 5.5	89.4 ± 5.1	68.0 ± 4.5	100.8 ± 7.1
Slide 3		50.5 ± 2.9	53.7 ± 3.8	35.8 ± 2.2	30.3 ± 1.6	40.2 ± 2.8
		119.9 ± 6.9	127.5 ± 9.0	85.0 ± 5.2	72.0 ± 3.8	95.5 ± 6.6
§ Average		110.8 ± 3.9	136.5 ± 4.0	89.1 ± 3.1	63.1 ± 2.7	100.6 ± 4.3
‖ Corrected average		100.0 ± 5.6	142.5 ± 7.1	109.4 ± 5.9	94.0 ± 6.6	123.0 ± 7.6

Geometry Control	**Hours	Uncorrected Interphase	‡‡Corrected Interphase	Prophase	Metaphase	Anaphase	Telophase
Slide 1	2.5	19.3 ± 1.5	21.7 ± 1.7	21.8 ± 1.5	18.7 ± 1.4	14.8 ± 1.6	14.8 ± 1.3
			118.2 ± 9.2	118.7 ± 8.2	101.8 ± 7.6	80.6 ± 8.7	80.6 ± 7.1
Slide 2	5.0	15.4 ± 1.2	19.4 ± 1.5	17.4 ± 1.5	16.5 ± 1.2	13.4 ± 1.4	17.3 ± 1.4
			115.5 ± 8.9	103.6 ± 8.9	98.2 ± 7.1	79.8 ± 8.3	103.0 ± 8.3
Slide 3	10.0	26.3 ± 1.8	41.7 ± 2.9	30.2 ± 1.7	22.6 ± 1.3	19.9 ± 1.3	27.6 ± 1.8
			146.8 ± 10.2	106.3 ± 6.0	79.6 ± 4.6	70.1 ± 4.6	97.2 ± 6.3
	Average		126.8 ± 5.5	109.5 ± 4.5	93.2 ± 3.8	76.8 ± 4.3	93.6 ± 4.2

* See Table I.
‡ See Table I.
§ See Table I.
‖ See Table I.
¶ See Table I.
** Hours in unlabeled medium after labeling.
‡‡ Correction was made for those cells which have gone through mitosis during the time in unlabeled medium.

TABLE III

Results of Whole Cell Grain Counts over Human Amnion Cells Exposed to Phenylalanine-C^{14} for 15 Minutes

Experiment		Interphase	Prophase	Metaphase	Anaphase	¶Telophase
Slide 1	*	119.2 ± 6.2	127.8 ± 7.0	55.2 ± 3.8	44.8 ± 2.6	64.2 ± 4.2
	‡	144.9 ± 7.5	155.4 ± 8.5	67.1 ± 4.6	54.5 ± 3.2	78.1 ± 5.1
Slide 2		138.6 ± 8.1	194.4 ± 7.6	71.0 ± 4.2	54.4 ± 2.7	84.0 ± 5.4
		127.8 ± 7.5	179.2 ± 7.0	65.4 ± 3.9	50.1 ± 2.5	77.4 ± 5.0
Slide 3		157.1 ± 11.0	199.8 ± 7.9	71.5 ± 4.7	58.2 ± 2.9	75.2 ± 5.0
		139.8 ± 9.8	177.8 ± 7.0	63.6 ± 4.2	51.8 ± 2.6	66.9 ± 4.4
§ Average		137.5 ± 4.8	170.8 ± 4.3	65.4 ± 2.4	52.1 ± 1.6	74.1 ± 2.8
‖ Corrected Average		100.0 ± 7.2	110.9 ± 6.2	47.9 ± 2.9	43.8 ± 3.2	52.5 ± 3.7

Geometry Control	**Hours	Uncorrected Interphase	‡‡Corrected Interphase	Prophase	Metaphase	Anaphase	Telophase
Slide 1	3	124.6 ± 7.8	133.5 ± 8.4	149.6 ± 7.6	132.8 ± 6.4	115.5 ± 7.6	137.0 ± 8.3
			99.9 ± 6.3	111.9 ± 5.7	99.3 ± 4.8	86.4 ± 5.7	102.5 ± 6.2

* See Table I.
‡ See Table I.
§ See Table I.
‖ See Table I.
¶ See Table I.
** See Table II.
‡‡ See Table II.

TABLE IV

Results of Whole Cell Grain Counts over Hamster Cells Exposed to Phenylalanine-C^{14} for 1 Hour or Uridine-C^{14} for 10 Minutes at End of Colcemide Treatment

Phenylalanine-C^{14}	**Hours	Interphase	C-Mitosis	Uridine-C^{14}	**Hours	Interphase	C-Mitosis
Slide 1	3 *	114.4 ± 6.5	81.1 ± 6.3	Slide 1	5.5	52.8 ± 3.8	2.6 ± 0.5
	‡	117.0 ± 6.6	83.0 ± 6.4			190.6 ± 13.7	9.4 ± 1.8
Slide 2	15	106.0 ± 9.1	48.0 ± 3.8	Slide 2	24.0	63.9 ± 7.1	2.0 ± 0.4
		137.7 ± 11.8	62.3 ± 4.9			193.9 ± 21.5	6.1 ± 1.2
Slide 3	30	118.4 ± 10.0	51.5 ± 4.6	Slide 3	39.5	66.2 ± 10.2	4.9 ± 1.5
		139.4 ± 11.8	60.6 ± 5.4			186.2 ± 28.7	13.8 ± 4.2
Slide 4	45	148.8 ± 12.8	76.7 ± 9.4				
		132.0 ± 11.4	68.0 ± 8.3				
§ Average (2, 3, and 4)		136.4 ± 6.7	§§ 63.6 ± 3.7	Average		190.2 ± 12.8	§§ 9.8 ± 1.6
‖ Corrected (average)		100.0 ± 6.6	76.8 ± 6.2	Corrected (average)		100.0 ± 6.7	8.5 ± 1.5

* See Table I.
‡ See Table I.
§ See Table I.
‖ See Table I.
¶ See Table I.
** Hours in colcemide.
§§ Anaphase geometry control was used as the correction for cells in C-mitosis.

early as prophase, the rate of RNA synthesis had fallen to 56 per cent of that of the average interphase value. By metaphase-anaphase, synthesis had fallen to a low of 13 to 16 per cent that of interphase. At telophase, the rate was increasing.

Phenylalanine Incorporation

HAMSTER CELLS: There is a significant rise in the incorporation of phenylalanine in prophase (42 per cent) over the average interphase rate. This period of high protein synthesis is followed by a fall at metaphase-anaphase to a rate not significantly different from the average interphase value. The results of the geometry controls indicate that the geometry of the anaphase hamster cell has a significant effect on the observed grain count of that phase. All data from these experiments are shown in Table II and Fig. 1.

HUMAN AMNION CELLS: Prophase appears to have the highest rate of protein synthesis, although the difference between it and the average interphase rate is not statistically significant when the three experiments are combined. At any rate, these cells show a significant drop in protein synthesis during metaphase-anaphase, at which time the rate is 52 to 56 per cent lower than the average interphase value. The geometry control data for the human amnion cells indicate that geometry does not significantly affect the grain counts. The data from these experiments are found in Table III and Fig. 1.

Colcemide Treatment

HAMSTER CELLS: Colcemide was employed to hold cells in a metaphase condition (C-mitosis) in order to test the effects of prolonged mitotic condition on uridine and phenylalanine incorporation in hamster cells. Phenylalanine-C^{14} was added to the medium for the last hour, or uridine-C^{14} for the last 10 minutes, of exposure (3 to 45 hours) to colcemide.

These cells cannot be held in C-mitosis indefinitely and eventually return to an abortive interphase condition with fragmented nuclei; this condition makes it possible to distinguish them from normal interphase cells. The interphase cells with fragmented nuclei accumulate steadily and are apparently incapable of reaching mitosis again. The length of time that cells remain in C-mitosis is somewhere between 3 and 15 hours, as judged from these experiments. The uptake of phenylalanine by cells treated with colcemide for greater than 3 hours was 77 per cent that of the average interphase. The uptake of uridine fell to 8 per cent of that of the average interphase rate. The data from these experiments are to be found in Table IV.

DISCUSSION

RNA Synthesis

The drop in uridine uptake observed in these experiments during normal and C-mitosis in the hamster cell line was much more drastic than any drop observed for phenylalanine uptake. Unlike the reports of other investigators (Feinendegen *et al.*, 1960; Taylor, 1960; Baserga, 1962 *b*; Prescott and Bender, 1962), however, these experiments showed RNA synthesis to be significantly above zero during mitosis.

RNA synthesis declined with the time spent in the mitotic condition and reached a very low level in the extended colcemide mitosis. The DNA of condensed mitotic chromosomes thus seems to be masked in such a way as to lower its ability to prime RNA synthesis. For example, the DNA-dependent RNA polymerase may not be able to bind to the DNA. Unless the cell is completely effective in masking its primer DNA, one might expect some new RNA to be made during mitosis. Also, it might be possible for some nucleolar material, although not visible, to still function in RNA synthesis.

Protein Synthesis

The high rate of phenylalanine incorporation found in prophase for both hamster and human amnion cells might be interpreted as an indication that the cell reaches its maximum rate of protein synthesis at that time. However, Prescott and Bender (1962) find no prophase increase in hamster cells when using histidine-H^3, and Baserga (1962 *a*), using leucine-C^{14} to label Ehrlich ascites tumor cells, finds protein synthesis to be maximal during the DNA synthetic period (S period).

The human amnion cells show a drop at metaphase-anaphase to about one-half of the interphase rate of phenylalanine incorporation. However, in the hamster cells, although there is a definite fall from the high rate at prophase, the metaphase-anaphase cells show no significant change from interphase. The hamster cell treated with colcemide is beginning to show a lowered rate of

protein synthesis, but there seems certainly to be no linear relationship between time in mitosis and drop in synthesis. Baserga (1962 *a*) and Prescott and Bender (1962) find the rate of amino acid incorporation to be lowered during mitosis, whereas Taylor (1960) finds amino acid incorporation unaffected by the division stage. It is difficult to explain the different results of Taylor, Prescott, and Bender, and those of this paper (all obtained from hamster cells). Prescott and Bender find a 75 per cent drop in histidine-H^3 incorporation during mitosis. One possible explanation of the differences between their results and those reported here involves the use of different isotopes. Due to the short range of tritium and the possible greater thickness of the mitotic cell relative to interphase cells, one may find the grain counts with tritium lower than those found using carbon-14 if no geometry correction is made.

The telophase rise in incorporation of both uridine and phenylalanine probably represents a transition period between the synthetic capacity of one cell and two cells. At some time after mitosis, the two daughter cells would be expected to show a combined rate twice that of the parent cell, but further experiments on both cell lines would be necessary to determine when this occurs.

Several differences between the patterns of RNA synthesis and protein synthesis are seen in these experiments: 1. The high rate of protein synthesis during prophase occurs at the same time that RNA synthesis has fallen drastically; 2. During mitosis, protein synthesis falls at most to one-half the average interphase value, while RNA synthesis is reduced to one-tenth; 3. Although protein synthesis would be expected eventually to cease under extended mitotic conditions, the results of the colcemide experiment indicate that, after several hours of extremely low RNA synthesis, protein synthesis is still relatively high. An interesting experiment of Goldstein, Micou, and Crocker (1960) showed that for 10 to 30 hours enucleated human amnion tissue culture cells are able to incorporate amino acids, but are unable to incorporate detectable amounts of RNA precursors.

The similar results found for mitotic and enucleated cells support Mazia's idea that the mitotic condition is in many ways similar to the enucleate condition (Mazia and Prescott, 1954). In both mitotic and enucleate cells, RNA synthesis is greatly suppressed while protein synthesis is affected much less. In the mitotic cell this condition lasts on the order of 10 minutes, with colcemide it can be extended to 3 hours, and in the enucleate cell it continues for as long as 30 hours. Unless normally the rate of messenger RNA synthesis is a very small fraction of the total RNA synthesis—so that the small rate of RNA synthesis in mitosis could represent a large fraction of normal messenger synthesis—the messenger RNA must have a half-life equal to or greater than 10 minutes in dividing cells, 3 hours in colcemide-treated cells, and 30 hours in enucleated cells. Levinthal, Keynan, and Higa (1962) find a much less stable messenger RNA in bacteria. However, the stability of messenger RNA might well vary among different cell types.

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