OXYGEN UPTAKE DURING THE HELA CELL LIFE CYCLE AND ITS CORRELATION WITH MACROMOLECULAR SYNTHESIS

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There have been several reports on the utilization of oxygen during the cell life cycle (1–7), with particular emphasis on the question of whether there is any change during mitosis. One of the most elegant of these studies, the "Cartesian Diver" experiments of Holter and Zeuthen (1) on synchronous egg cells, indicated that the oxygen requirement was decreased in mitosis. While Scholander et al. (2, 3) have challenged these results, earlier work of Erickson (4), and Stern and Kirk (5) on synchronous microspores of *Lilium* and *Trillium*, respectively, confirm them; and

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Gershenson et al. (6) have noted the same for HeLa cells synchronized with amethopterin. Swann (7) has suggested that the cell needs less oxygen during mitosis because of an "energy reservoir" built up prior to prophase. This hypothesis was consistent with data from many laboratories that showed oxygen deprivation, uncoupling agents, and various other metabolic inhibitors were ineffective in preventing cell division once it had begun, but did stop cell division when added premitotically. Gershenson et al. (6) reported that the nucleoside triphosphate content of HeLa cells reaches a maximum just before mitosis, and that the minimum coincides with a peak in the mitotic index, consistent with the "energy reservoir" hypothesis. Gelfant (8), however, has seriously questioned this concept and has suggested that any drop in nucleoside triphosphate levels might be explained by a decreased energy need during division, rather than by the emptying of a previously filled reservoir. This is consistent with data obtained in this laboratory on HeLa cells, which show that although mechanical work is presumably expended at division there is no RNA or DNA synthesis, and that protein synthesis as well as phospholipid synthesis are less than 50% of interphase levels (9). Likewise, Plesner has interpreted the increase in predivision ATP and GTP in *Tetrahymena* as evidence of decreased utilization (10). The present study confirms earlier reports of a decreased oxygen requirement during mitosis in the HeLa cell and shows that the drop in macromolecular synthesis at that time is adequate to account for the decreased oxygen need. Data are presented indicating that during peak DNA synthesis about 80% of the cell's oxidative energy is expended on macromolecular synthesis, whereas during mitosis only 7% is utilized for macromolecular synthesis, and that about 17% goes toward the work of cell division. Although these results are in contrast with the *lack* of correlation between O_2 and macromolecular synthesis found by Polgar et al. in phytohemagglutinin-stimulated cells (11), it is likely that the discrepancy is due to the significant difference between lymphocytes and HeLa cells.

MATERIALS AND METHODS

HeLa cells (S_3 strain) were maintained as monolayers or in spinner culture as previously described (9). Cells were synchronized by the following two methods: (*a*) Optimal synchrony of cells in S was obtained by placing cultures in spinner medium containing 2 mM thymidine for 12 hr followed by replacement with normal medium (12); a sharp peak of DNA synthesis occurred predictably 3½ hr later. (*b*) In order to obtain populations almost all of which were in mitosis, thymidine-treated spinner cultures were converted to monolayer cultures in low calcium medium, and mitotic cells were selectively detached from the monolayers by gentle rocking, 10 hr after removal of the thymidine (9). These cells that were placed in spinner culture completed mitosis without lag and remained well synchronized through G_1. In some experiments, colchicine (0.1 mg/ml) was added to the monolayers. Pure metaphase-arrested populations were then obtained by selective detachment 2 hr after addition of the drug and compared with normally dividing cells with respect to oxygen uptake.

Macromolecular synthesis in synchronized cells was assayed by incorporation of uridine-^{14}C thymidine-^{14}C, or ^{14}C-labeled amino acids into trichloroacetic acid-precipitable material, and of $^{32}PO_4$ into the chloroform-methanol (2:1) extractable fraction as already described (9). Parallel measurements of oxygen uptake were made with a Model 53 Biological Oxygen Monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio). 3-ml aliquots of synchronized cells at concentrations of 1–3 mg/ml were withdrawn from the spinner at appropriate intervals, placed in the electrode stirring compartment, and oxygen uptake measurements were made for 10-min periods at 37°C. 10-ml aliquots were simultaneously withdrawn, and packed cell mass was measured by microhematocrit as previously described (13). The effects of inhibitors of RNA, DNA, and protein synthesis on oxygen consumption were determined either by adding the drugs directly to the cells in the electrode stirring compartment and noting the immediate effects or by adding them to the stock cultures and removing aliquots at appropriate intervals. Actinomycin D (Merck & Co., Inc.; New York) (2 μg/ml), cytosine arabinoside (Sigma Chemical Co., St. Louis, Mo.) (40 μg/ml), and Acti-dione (Mann Research Laboratories, Inc., New York) (60 μg/ml) were used to inhibit RNA, DNA, and protein synthesis, respectively.

RESULTS AND DISCUSSION

HeLa cells synchronized with 2 mM thymidine show a good correlation between DNA synthesis and oxygen uptake. There is a sharp peak of oxygen uptake occurring 3½–4 hr after removal of the excess metabolite, followed by a less rapid decline as the cells complete S (DNA synthetic period) and pass through G_2 (postDNA synthetic period) and mitosis (Fig. 1). Oxygen uptake approaches a minimum value as cells enter mitosis; however, it is difficult to ascertain the precise low

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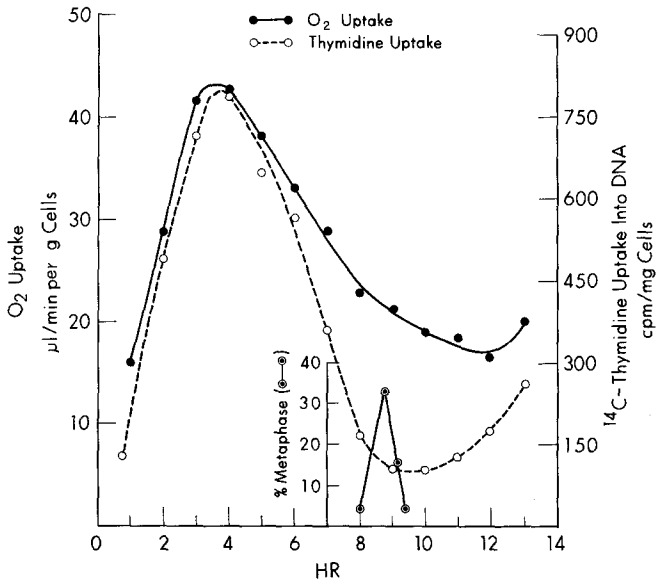

FIGURE 1 Curves showing relationship of oxygen uptake to thymidine-^{14}C uptake in cells synchronized with 2 mM thymidine. Cells maintained at a concentration of 1–3 mg/ml were exposed to 2 mM thymidine for 12 hr and then were placed in fresh spinner medium. 3-ml aliquots were removed at indicated points and assayed for oxygen uptake. 1-ml aliquots were removed at the same time and pulsed for 15 min with 0.5 µc of thymidine-^{14}C. Samples were washed, precipitated with 5% trichloroacetic acid, filtered onto millipore discs, dried, and counted in a low background gas flow counter (9).

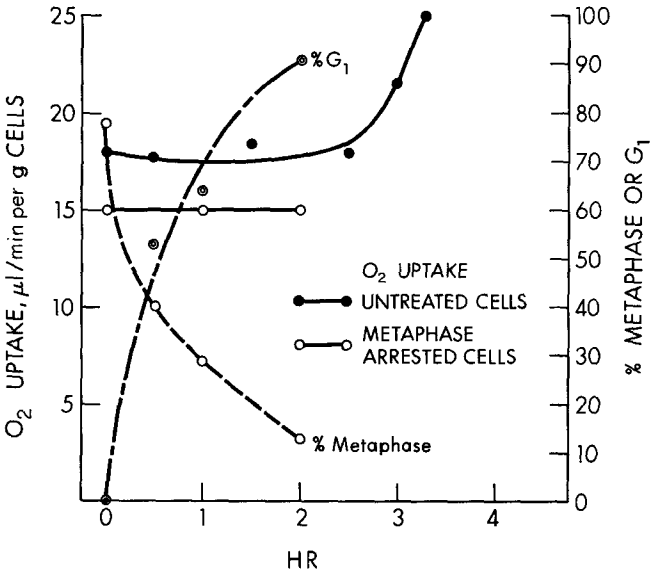

FIGURE 2 Curves showing oxygen uptake in populations synchronized by selective detachment of mitotic cells from monolayers grown in low Ca^{++} medium. Assays were carried out as in Fig. 1. Oxygen uptake in metaphase-arrested population is plotted on the same ordinates. These cells were collected by selective detachment of mitotic cells from monolayer cultures treated for 2 hr with colchicine (0.1 µg/ml).

point in this type of population because thymidine-synchronized cells have partially lost their synchrony by the time they reach mitosis. In general, the mitotic index never exceeds 35%, and consequently an accurate appraisal of oxygen uptake at this stage cannot be made. When essentially pure populations of mitotic cells are obtained by selective detachment from monolayers, it is found that the average oxygen uptake is at least as low in early G_1 (preDNA synthetic period) as it is during mitosis (Fig. 2). In occasional experiments, levels during early G_1 are even lower than in mitosis (see below). Uptake then rises as the cells proceed toward S. Fig. 2 also shows that oxygen utilization by cells arrested in metaphase with colchicine is about 17% below that of untreated metaphase cells and remains constant for 2 hr. Although not shown here, colchicine was found to have no effect on oxygen uptake by *interphase* cells. Therefore, the difference between metaphase-arrested and

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untreated mitotic cells does not appear to be a drug-induced artifact and may be related to the work expended by the untreated cell in the act of division, as is discussed below.

In order to estimate the relative contribution of macromolecular synthesis to oxygen uptake during the cell cycle, various metabolic inhibitors were added and their effects noted (Table I). Cytosine arabinoside added at the peak of DNA synthesis caused a drop of 34% in oxygen uptake, which is a surprisingly large fraction of the cell's total consumption even when the concomitant arrest of histone synthesis (14) is taken into consideration. There was no change when the inhibitor was added during metaphase or early G_1 at which point there is no DNA synthesis. Acti-dione (cycloheximide) lowers oxygen uptake by 62% when added at the peak of S. Besides arresting protein synthesis, this drug is known to arrest DNA replication as well, perhaps by preventing histone synthesis. The contribution of nonhistone protein synthesis to oxygen uptake at the peak of S may, therefore, be estimated to be 28% of the total (62% − 34%). Finally, actinomycin D, which at the concentration used inhibits 90% of RNA synthesis, causes a 19% drop in oxygen consumption during peak S and only a 2% and 8% decrease during metaphase and early G_1, respectively, at which times there is very little RNA synthesis. We have found that the effects of the various metabolic inhibitors reach a maximum within 5 min after their addition and that the new slope is maintained for the 20-min duration of the experiment. These results tend to rule out the possibility that the decline in oxygen consumption caused by inhibitors is due to nonspecific toxicity, as this would presumably be progressive.

The postulated work of cell division coupled with the contribution of macromolecular synthesis to oxygen uptake allows a reasonable explanation for the curves shown in Figs. 1 and 2, particularly

TABLE I

Effects of Inhibitors of Macromolecular Synthesis on O_2 Uptake by Monodisperse HeLa Cells

Phase of cell cycle	% inhibition of total O_2 uptake		
	Cytosine arabinoside	Acti-dione	Actinomycin D
Peak S	34±1*	62±2	19+1
Metaphase	0	5±<1	2±<1
Early G_1	0	19±<1	8±<1

* Standard error for four experiments.

Cells arrested in metaphase with colchicine and synchronized cells at the peak of S or in early G_1 were treated with the indicated inhibitors and oxygen uptake was compared to that of untreated controls. Inhibitor concentrations were: cytosine arabinoside (40 μg/ml), Acti-dione (60 μg/ml), and actinomycin D (2 μg/ml).

TABLE II

Macromolecular Synthesis in Monodisperse HeLa Cell Cultures at Different Phases of the Cell Life Cycle

Precursor	% peak S			
	Mitotic	Metaphase arrest	Early G_1	Peak S
Thymidine-^{14}C	3	4.4	10	100
Uridine-^{14}C	7	10.0	30	100
^{14}C-labeled amino acids	37	33.0	49	100
$^{32}PO_4$-($CHCl_3$: CH_3OH extract)	37	31.0	49	100

Synchronized cells in mitosis, metaphase arrest, early G_1, and S (peak) were assayed for macromolecular synthesis by noting uptake of the following indicated precursors into trichloroacetic acid-precipitable material: thymidine-^{14}C (0.5 μc/ml), uridine-^{14}C (0.1 μc/ml), ^{14}C-labeled amino acids (0.1 μc/ml), $^{32}PO_4$ (20 μc/ml). At the peak of S cpm were taken as 100%, and counts at all other times were compared to this peak value. Appropriate corrections were made for mass changes.

TABLE III

Contribution of Various Cellular Functions to O_2 Uptake in the HeLa Cell

Cell function	O_2 Uptake		
	Peak S	Mitotic	Early G_1
	μl/min per g cells		
DNA synthesis	14.0	0*	0*
Protein synthesis	12.0	0.9	3.4
RNA synthesis	8.0	0.4	2.0
Cell division	0.0	3.1	0.0
Remainder	9.5	13.7	12.0
Total	43.5	18.1	17.4

Calculated from data in Tables I and II and Figs. 1 and 2.

* Unmeasurable with the techniques used.

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the constancy of the uptake curve from mitosis through early G_1. At this latter time, the division has been completed, but as can be seen in Table II, the curtailment of macromolecular synthesis that occurs during mitosis has not yet been fully reversed, i.e. the difference between the uptake of macromolecular precursors in mitotic cells and G_1 cells is existent but considerably less than that seen when cells in G_1 are compared with those in S (protein, RNA, and phospholipid synthesis levels in S are more than twice the levels in G_1, while DNA synthesis is at least 10 times higher). Therefore, it is proposed that the oxygen uptake required for the work of division in the mitotic cell is about equal to that required for the moderate postmitotic increase in macromolecular synthesis occurring in the G_1 cell, with the result that the total oxygen uptake remains unchanged during this time. The lower uptake levels obtained with metaphase-arrested cells may provide for a quantitative assay of the oxygen uptake expended on division. In Table III, it is indicated that this may amount to about 3.1/18 or 17% of the total.

The relative contribution of oxidative metabolism to various other cell functions in mitosis is also shown in Table III. At the peak of S about 80% of the oxidative energy is utilized for synthesis of RNA, DNA, and protein with the remaining 20% presumably contributing to functions such as sodium and potassium transport, lipid metabolism, etc. On the other hand, during mitosis less than 10% of the total oxidative energy expended goes toward macromolecular synthesis. It is assumed that this marked decrease is a function of the morphological transitions that characterize this period in the life cycle. Thus, nucleolar dispersion and chromosome condensation presumably preclude RNA transcription; polyribosome disaggregation depresses protein synthesis and the earlier completion of replication of the genome prohibits further DNA synthesis.

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REFERENCES

1. HOLTER, H., and E. ZEUTHEN. 1957. *Publ. Staz. Zool. Napoli.* **29**:285.
2. SCHOLANDER, P. F., C. L. CLAFF, S. L. SVEINSSON, and S. I. SCHOLANDER. 1952. *Biol. Bull. (Woods Hole).* **102**:185.
3. SCHOLANDER, P. F., H. LEIVESTAD, and G. SUNDNES. 1958. *Exp. Cell Res.* **15**:505.
4. ERICKSON, R. O. 1947. *Nature (London).* **159**:275.
5. STERN, H., and P. KIRK. 1948. *J. Gen. Physiol.* **31**:243.
6. GERSHENSON, L. E., F. F. STASSER, and D. E. ROUNDS. 1965. *Life Sci.* **4**:927.
7. SWANN, M. M. 1957. *Cancer Res.* **17**:727.
8. GELFANT, S. 1959. *Exp. Cell Res.* **16**:527.
9. ROBBINS, E., and M. D. SCHARFF. 1966. *In* Cell Synchrony—Studies in Biosynthetic Regulation. I. Cameron and G. Padilla, editors. Academic Press Inc., New York. 353.
10. PLESNER, P. 1964. *In* Synchrony in Cell Division and Growth, E. Zeuthen, editor. Interscience Publishers Inc., New York. 197.
11. POLGAR, P. R., J. M. FOSTER, and S. R. Cooperband. 1968. *Exp. Cell Res.* **49**:231.
12. BOOTSMA, D., L. BUDKE, and O. VOS. 1964. *Exp. Cell Res.* **33**:301.
13. MORRILL, G. A., and E. ROBBINS. 1967. *J. Gen. Physiol.* **50**:781.
14. ROBBINS, E., and T. BORUN. 1967. *Proc. Nat. Acad. Sci. U. S. A.* **57**:409.

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