EFFECTS OF DIVISION-SYNCHRONIZING HYPOXIC AND HYPERTHERMIC SHOCKS UPON *TETRAHYMENA* RESPIRATION AND INTRACELLULAR ATP CONCENTRATION

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ABSTRACT

The division of *Tetrahymena pyriformis* GL cells was synchronized with either seven hypoxic or five hyperthermic (heat) shocks. Hyperthermic shocks of 34°C produced no reduction in respiration rate and only a 19% decline in intracellular ATP concentration. Hypoxic shocks of 0.15% ambient oxygen concentration depressed intracellular ATP concentration 50%. It therefore appears that hypoxic shock, but not hyperthermic shock, reverses progress of *Tetrahymena* toward fission by reducing ATP concentration through a reduction of the rate of oxidative phosphorylation. After the first synchronized division, whether synchronized by intermittent hypoxia or hyperthermia, total respiration rate increased exponentially at the same rate of increase as total respiration rate in an exponentially growing (log phase) *Tetrahymena* cell culture. Before the first synchronized division, the total respiration rate increased exponentially but more slowly than after completion of the first synchronized division. The pattern of increase of total respiration during division synchron*ized* by either procedure was different than the pattern of increase of total respiration of synchron*ous* cells observed by Zeuthen.

INTRODUCTION

Investigators have developed various techniques to synchronize cell division in order to facilitate studies of the sequences of biochemical events which occur during the division cycle. Division synchronization provides a form of metabolic "amplification." It is far easier to isolate and identify the chemical constituents from several million synchronized cells at each stage of the division cycle than from a single cell.

Division synchronization in cell cultures may be achieved with temporary environmental changes which *selectively* and reversibly block one stage of the division cycle or which reverse progress beyond one stage of the division cycle. While providing metabolic amplification, synchronizing environmental manipulations *can* produce metabolic "distortion" as in the case of artificial (1, 18) phasing of DNA synthesis in *Escherichia coli* [15]T-synchronized by removal and restoration of thymine to the culture medium (6). The introduction of metabolic distortion is undesirable if one wishes to follow the biochemical events in a cell which enters division free of metabolic perturbation.

One approach to trying to determine which metabolic patterns attend an "unperturbed" division is to carry out studies on cells synchronized by more than one method. According to this approach, if the same pattern of biochemical change occurs during the synchronized division of a cell population, regardless of the type of synchronizing procedure employed, then that pattern of biochemical change is assumed likely to occur during the division of an unperturbed cell. This "alternate procedure approach" was taken by Moner and Berger who studied the temporal pattern of RNA synthesis during the division of *Tetrahymena pyriformis* cells synchronized in one study (19) by periodic elevations of temperature and in another study (21) by periodic reductions of temperature.

With the intention of employing the alternate procedure approach to studies of protozoan cell division, we have developed an additional technique for synchronizing *Tetrahymena* cells, i.e. the utilizing of intermittent hypoxia (25) applied with an automatic apparatus (26). It was our intention to carry out studies of the biochemical patterns which accompany the division of *Tetrahymena* synchronized by two alternate procedures, periodic elevations of temperature and periodic reductions of oxygen concentration. However, before carrying out such biochemical studies, we have carried out studies (reported here) on two aspects of the use of intermittent hypoxia and intermittent hyperthermia as alternate synchronizing procedures.

First, we wanted to know if the two procedures differed enough in their effect upon cells *during the time of application* to be considered radically different synchronizing methods. To see if the hypoxic and hyperthermic procedures differ in their effect upon *Tetrahymena* cells during the time of application, we have compared the effects of both types of shocks upon intracellular ATP concentration. We have also carried out measurements to determine if respiration is significantly depressed by hyperthermic shock (we assume that respiration is depressed during hypoxic shock for the reasons presented in the Discussion).

Second, we have put the alternate procedure approach to a test. The pattern of respiration in synchro*nous* (unperturbed) *Tetrahymena* cells has already been determined by Zeuthen (32). We have followed the patterns of respiration in *Tetrahymena* cells synchroni*zed* by both procedures to see if conclusions about respiration based upon the alternate procedure approach are correct. We wanted to see if *both* the hypoxic and hyperthermic

synchronizing procedures would distort the pattern of respiration, and if so, to see if the distortions are the same in both cases. If the distortions are the same, then the alternate procedure approach would lead (in the absence of Zeuthen's data) to an incorrect impression about the respiration pattern in unperturbed cells.

MATERIALS AND METHODS

Synchronizing Division with Hypoxic and Hyperthermic Shocks

The programs of intermittent hypoxia and intermittent hyperthermia and their application to *Tetrahymena* cultures have been previously described in detail (26), as has the preparation of the culture medium (25, 26). Briefly, *Tetrahymena pyriformis* GL cells in exponential phase at a population density between 30 and 40 thousand cells/ml in a proteose-peptone, liver-extract medium were synchronized with either five 20-min 34°C periods alternated with four 40-min 28°C periods or seven 45-min exposures to 0.15% (by volume) oxygen alternated with 45-min exposures to air at 29°C. The fifth hyperthermic shock is terminated by transferring the culture flask to a Dubnoff bath at 29°C.

Determining Cell Number

1 ml samples were withdrawn from the culture flasks, diluted with counting solution, and counted on the Coulter counter model B as described in reference 26.

Determining Mean Cell Volume

In order to determine the effects of each type of shock upon intracellular ATP concentration, measurements were made of changes in average cell volume during these shocks. The Coulter particle size distribution plotter, attached to the model B, was utilized at settings of aperture current^{-1} of 0.707, amplification^{-1} of 32, matching switch at 32L, gain of 100, cycle time of 8 sec. Measurements were carried out upon 3-ml *Tetrahymena* cell culture aliquots diluted with 25 ml of counting solution. A Fortran 4 program was devised to compute the mean cell size on a linear plotter scale from each of the histograms produced by the Coulter particle size distribution plotter. The program assigns a plotter scale value of s-$\frac{1}{2}$ units to all cells falling into a given size category s of a histogram.

The Coulter plotter was calibrated with the use of a protozoocrit (10, 28). A 200 ml suspension culture of 92.6 thousand cells/ml was subjected to two hypoxic shocks and then to successive hyperthermic shocks. After the second hypoxic shock and succeeding hyperthermic shocks, mean cell volume determina-

tions were made upon 10-ml aliquots with a proto-zoocrit. Simultaneously, 3-ml aliquots were analyzed with the Coulter plotter. In agreement with theoretical predictions (8, 14), Fig. 1 shows that mean *Tetrahymena* cell volume is proportional to the mean size on the Coulter plotter scale. It should be noted, however, that small volumes of solution between packed cells in a protozoocrit may tend to offset the calibration slightly.

Measuring Respiration

Measurements of the rate of oxygen consumption of *Tetrahymena* cells were carried out in a shallow, large diameter (54 mm) Warburg flask. Respiration measurements were performed upon *T. pyriformis* GL cells under four conditions as described below.

First, respiration measurements were performed upon a 5 ml cell sample as it underwent hyperthermic synchronization. The automatic temperature bath of Lee (15), normally used for hyperthermic synchronization, was not used for this respiration study because small temperature drifts made precise measurements impossible. Instead, an insulated automatic temperature bath, designed by Schmid and Eiler and described by Ashley (3), was used. Since this bath required 10 min for both the 28°–34°C transition and the return to 28°C, a 22½ min shock/37½ min growth cycling program was chosen so that a 10 min interval of respiration could be reliably measured at 34°C and so that the cells would spend 12½ min of each 1 hour cycle *at* 34°C as in the case of the 20 min shock/40 min growth program on the automatic temperature bath of Lee which required only 7½ min to make the 28°–34°C transition. The manometer stopcocks were opened at the beginning and closed at the end of each programmed shift in temperature, 2 min

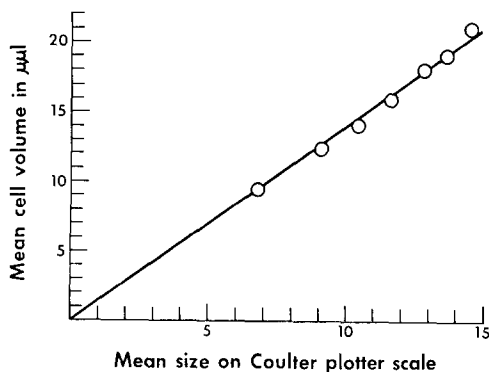**Mean size on Coulter plotter scale**

FIGURE 1 Proportionality between mean *Tetrahymena* cell volume as determined by a protozoocrit and mean size on the Coulter plotter scale. The ordinate represents packed volume/cell in a protozoocrit. The abscissa represents mean size in a histogram produced by the Coulter plotter.

Period	Normalized[*2] total respiration
First shock period	$6.1 \times 10^{-8} \mu l\ O_2/$ sec
First growth period	7.9
Second shock period	10.6
Second growth period	10.2
Third shock period	9.5
Third growth period	10.6
Fourth shock period	9.8
Fourth growth period	11.6
Fifth shock period	12.7

There were 31.2 thousand cells in the Warburg flask at the end of the fifth hyperthermic shock.
* 5-ml aliquots were taken from the same hypoxically synchronized cultures whose cell numbers are plotted in Figs. 3 and 4 of reference 26.

after the stopcocks had been closed, a set of manometer readings was made. At 34°C a second set of readings was made 10 min after the first. At 28°C a second set of readings was made 25 min after the first. Total respiration was calculated, for each interval, in terms of microliters of O_2 per second consumed and then "normalized"[1] by dividing by the number of cells present in the sample flask at the end of the fifth hyperthermic shock. The results of these calculations are presented in Table I.

Second, the respiration of a sample from an exponential phase *Tetrahymena* cell culture was measured periodically in the Warburg flask in an insulated Aminco bath at 29°C. At time zero, in Fig. 3, a 5 ml sample was withdrawn from a 55 ml exponential phase culture in a 1 liter Erlenmeyer flask. Over a period of approximately 4 hr, cell counts were made on the contents of the Erlenmeyer flask. During the same 4 hr period the respiration rate of the Warburg contents was periodically measured over 30-min intervals. The total respiration during each interval was normalized by dividing by the number of cells present in the Warburg flask at time zero.

Third, measurements were made of the respiration of *Tetrahymena* cells which had undergone seven division-synchronizing hypoxic shocks. In duplicate experiments,[2] 30 sec after termination (beginning of air inflow) of the seventh shock, 5-ml aliquots of synchronized cell culture were transferred to the Warburg flask. 5 min after termination of the shock,

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[1] See the last paragraph under *Measuring Respiration* in Materials and Methods.

[2] 5-ml aliquots were taken from the same hypoxically synchronized cultures whose cell numbers are plotted in Figs. 3 and 4 of reference 26.
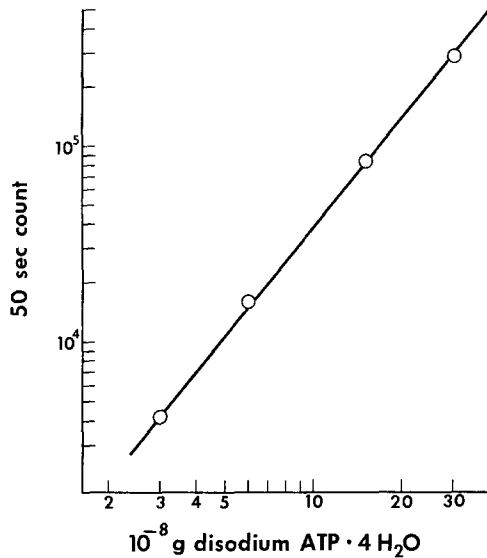
FIGURE 2 Logarithmic relation between ATP and scintillation spectrometer count during the firefly luminescence reaction. The ordinate represents the value of a count compiled by a Packard 3003 spectrometer between 20 and 70 sec after the start of the firefly luminescence assay reaction for ATP. The abscissa represents the quantity of ATP in the 1 ml sample added to the 1 ml of dissolved Worthington firefly extract in a liquid scintillation vial. The assay reaction was carried out at 0°C.

the first of 25 sets of manometer readings, spaced at 10-min intervals, was taken. Total respiration during each interval was normalized by dividing by the number of cells present at the end of the seventh shock. The results of the duplicate experiments were averaged for presentation in Fig. 3.

Fourth, measurements were made of the respiration of *Tetrahymena* cells after they had undergone five division-synchronizing hyperthermic shocks. In duplicate experiments,[3] 5-ml aliquots of cell culture were transferred to the Warburg flask just before the fifth shock. Each sample was subjected to the fifth hyperthermic shock in the automatic temperature bath of Lee. The fifth hyperthermic shock was terminated by transfer of the Warburg flask to the 29°C Aminco bath. 5 min after termination of the shock the first of 24 sets of manometer readings, spaced at 10-min intervals, was taken. The results were normalized and averaged for presentation in Fig. 3.

[3] One 5 ml aliquot was taken from the same hyperthermically synchronized culture whose cell numbers are plotted in Fig. 3 of reference 26. The other 5 ml aliquot was taken from another cell culture synchronized in the same manner.

It should be noted that normalization of respiration rate involved only division of respiration rate by the number of cells in the Warburg flask at the end of the last synchronizing shock or when taken from exponential phase cell culture. This was not normalization in the strict mathematical sense but was merely a division carried out to equalize total respiration and respiration per cell over part of the period in which measurements were made. In the case of respiration measurements *during* hyperthermic synchronization, normalized total respiration equaled respiration per cell from the beginning of the second shock until the end of the fifth shock, a period when cell number remains constant. In the case of respiration measurements made *after* synchronization, normalized total respiration equaled respiration per cell until 80 min after termination of the final shock for hyperthermically synchronized cells and until 100 min after termination of the final shock for hypoxically synchronized cells. In the case of measurements of respiration of the exponential phase culture, normalized total respiration equaled respiration per cell only at the time of transfer of the 5 ml aliquot to the Warburg flask. A more ideal normalization might be a division by total cell volume or total number of mitochondria.

Measuring Intracellular ATP

Measurements of adenosine triphosphate in small aliquots of *Tetrahymena* cell suspensions are now feasible with the use of the liquid scintillation spectrometer. Tal et al. (29) and Addanki et al. (2) found that the Packard spectrometer is so sensitive to the firefly luminescence assay reaction for ATP that, even at 0°C, ATP can be quantitated in the 10^{-10}–10^{-12} mole range. Because such small quantities of ATP can be measured, there is no need to collect a large number of cells by cold centrifugation as was the case in previous attempts to assay ATP in *Tetrahymena* (9, 27). During the lengthy process of chilling and compacting, it seems reasonable to expect significant changes in the content of ATP, the nucleotide moiety of which, in synchronized *Tetrahymena*, was estimated by Plesner (23) to turn over at a rate of approximately 240 times per minute. The ATP content would remain constant only if the metabolic pathways involved in the biosynthesis and the degradation of ATP display the same temperature dependence and sensitivity to hypoxia in the compacted pellet. Accordingly, ATP was extracted from small uncentrifuged samples of *Tetrahymena*, and the neutralized cold perchloric acid extracts were then assayed with the firefly luminescence reaction in the Packard spectrometer, according to the procedure described below.

The procedure used to assay ATP in *Tetrahymena* cell suspensions is as follows. Samples of 3 ml each
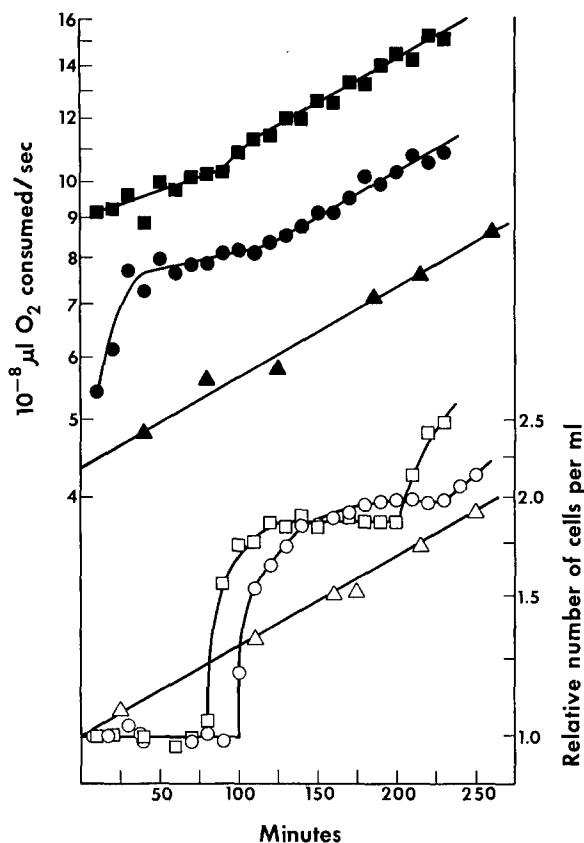
FIGURE 3 Increase in total respiration and relative cell number during exponential phase (unsynchronized) *Tetrahymena* cell division and after hypoxic and hyperthermic synchronization procedures. The left ordinate represents normalized[2] total respiration during exponential phase cell division, ▲; after hypoxic division synchronization, ●; after hyperthermic division synchronization, ■. The right ordinate represents relative cell number during exponential phase cell division, △; after hypoxic division synchronization, ○; after hyperthermic division synchronization, □. A relative cell number of 1.0 is equivalent to 38.4 thousand cells/ml in the case of exponential cell division, an average of 39.6 thousand cells/ml in the case of hypoxically synchronized cell division, an average of 33.6 thousand cells/ml in the case of hyperthermically synchronized cell division. The abscissa represents the time elapsed after transfer of a 5 ml aliquot of exponential phase cell culture to the Warburg flask and the time elapsed after the termination of each synchronization procedure.

were removed from the culture flask. Each sample was quickly emptied into a flask which contained 5 ml frozen 1 N perchloric acid plus 2 ml cold liquid 1 N perchloric acid. After about 10 min in an ice-water slurry, the flask (with Parafilm cover, American Can Company, Neenah, Wis.) was then stored at −15°C for no longer than 3 days. After storage, the thawed, cold contents were centrifuged at 0°C, and the supernatant was filtered. 5 ml of the filtrate were transferred to a graduated conical tube containing ½ ml of 4% glycyl glycine buffer. Cold potassium hydroxide solution was carefully added, and the course of the neutralization was followed with a pH meter. After pH 7.4 was reached, the contents were brought to 10 ml volume with the addition of cold distilled water, restirred, and placed on ice to allow the potassium perchlorate to settle. The supernatant was stored at −15°C and was ready for assay with the firefly luciferin-luciferase system upon thawing. ATP standards were prepared in the same manner except that 3-ml samples of cell-free medium were utilized without the centrifugation and filtration steps. Discrete quantities of aqueous Mann Assayed disodium ATP·4H₂O were added to the contents of

the graduated conical tube before adjusting to pH 7.4.

The prepared ATP samples were assayed with Worthington firefly extract (Worthington Biochemical Corp., Freehold, N.J.), a source of the luciferin-luciferase system. To the contents of each vial of firefly extract 25 ml of cold distilled water were added. This was then filtered. 1 ml aliquots were placed in Parafilm-covered, glass, scintillation vials. These were kept on ice, as were the thawed, deproteinized ATP samples.

A Packard model 3003 scintillation spectrometer was used to record the rate of photon pair emission during the assay reaction. The spectrometer's first channel was used at settings of 50% gain, 50–1000 discriminator range. In the case of each ATP sample, a 1 ml portion was transferred, at time zero, to one of the vials containing 1 ml dissolved firefly extract. At time +20 sec a 50 sec count was begun. The 50 sec counting period was observed to include the interval of maximum rate of photon emission. As may be seen in Fig. 2, the 50 sec count and the amount of ATP in the sample which had been added to the scintillation vial exhibited a logarithmic rela-

tionship. This logarithmic response contrasts with the linear response observed by Addanki et al. (2) under the conditions of their ATP assay. With every set of samples two ATP standards were run to establish the position of the log-log line.

A test was run to determine the relative sensitivity of our luciferase assay to ATP and ADP. The assay was shown to be 42.5 times more sensitive to ATP when a 4.84×10^{-10} mole ADP sample registered counts equivalent to $4.84 \times 10^{-10}/42.5$ mole ATP. No test was carried out to determine the sensitivity of the assay to guanosine triphosphate (GTP), which various authors have found to undergo the luciferase luminescence reaction to a limited degree. Plesner (23) found that most of the nucleoside triphosphate in synchronized Tetrahymena is in the form of ATP and that changes in GTP content paralleled the changes in ATP content.

The ATP measured was shown to be intracellular by assay of the medium from which Tetrahymena cells had been removed by centrifugation. Such samlples, obtained before and after both hypoxic and hyperthermic shocks, registered negligible counts of less than 40.

Determinations of Tetrahymena ATP were found to be reproducible at each point within a variation range of ±15%. ATP determinations were carried out at the onset, 20 min after the onset of the second, third, and fourth hyperthermic shocks, and at the onset (seconds before the inflow of nitrogen began), 20 min after the onset, and 44 min after the onset of the third, fourth, and fifth hypoxic shocks. These determinations were carried out upon six separate Tetrahymena cultures, three undergoing hypoxic shocks, three undergoing hyperthermic shocks. The ATP values in Table II represent the median of three determinations at each point.

RESULTS

As indicated in Table I, there was no observable depression of the rate of respiration during individual 34°C hyperthermic shocks. As Table II indicates, 0.15% oxygen shocks depressed mean intracellular ATP concentration approximately 50%. On the other hand, mean ATP concentration declined only 19%, during the course of individual hyperthermic shocks, to a value still 13%

TABLE II

Effect of Hypoxic and Hyperthermic Shocks upon the Intracellular ATP Concentration of Tetrahymena cells

Situation	ATP content	Cell volume	ATP concentration
Third hypoxic shock			
at the beginning	2.180×10^{-15} mole	1.130×10^{-11} 1	1.930×10^{-4} M
after 20 min	0.985	1.091	0.904
after 44 min	0.918	0.860	1.068
Fourth hypoxic shock			
at the beginning	1.799	1.215	1.481
after 20 min	0.965	1.150	0.839
after 44 min	0.697	1.038	0.672
Fifth hypoxic shock			
at the beginning	2.418	1.328	1.820
after 20 min	1.143	1.278	0.894
after 44 min	1.070	1.133	0.944
Average at the beginning of hypoxic shock			1.765
Average 20 min after the beginning of hypoxic shock			0.879
Average 44 min after the beginning of hypoxic shock			0.894
Second hyperthermic shock			
at the beginning	2.428	1.119	2.170
after 20 min	2.186	1.155	1.893
Third hyperthermic shock			
at the beginning	3.717	1.277	2.910
after 20 min	2.497	1.410	1.773
Fourth hyperthermic shock			
at the beginning	3.810	1.634	2.333
after 20 min	3.748	1.625	2.309
Average at the beginning of hyperthermic shock			2.471
Average 20 min after the beginning of hyperthermic shock			1.991

higher than immediately before the onset of individual hypoxic shocks and 124% higher than the value during individual hypoxic shocks.

In Fig. 3 it is shown that, in the cell population densities employed for these studies, respiration rate per cell remained constant (at approximately $4.4 \times 10^{-8} \mu l$ O_2/sec) in the case of an exponentially growing culture. Respiration rate per cell increased during the course of both hypoxic and hyperthermic synchronization treatments. As may be seen in Fig. 3, hypoxically synchronized cells required approximately 30 min after the seventh shock to reestablish a rapid rate of respiration. After this recovery, the total respiration rate increased exponentially[4] with time with an increase of exponential rate constant[4] after most of the cells had completed fission. Total respiration also increased exponentially before and after fission of hyperthermically synchronized cells, with the exception of a slight nonexponential rise just after most of the cells had completed the first synchronized division. In the case of both hypoxically and hyperthermically synchronized cells, total respiration increased more slowly (smaller exponential rate constant) before fission than after. After fission, total respiration increased exponentially with the same exponential rate constant as that of an unsynchronized *Tetrahymena* cell culture.

DISCUSSION

Hypoxic and Hyperthermic Shocks

Measurement of respiration rate of *Tetrahymena* cells during hypoxic shock was not undertaken because account was taken of the difficulty of maintaining in the Warburg flask the conditions of surface/volume, agitation, and oxygen concentration which exist in the large flask (26) during hypoxic shock. Therefore, hypoxia was assumed to considerably reduce respiration rate on the basis of three findings. First, a Michaelis-Menten relation between oxygen concentration and respiration was found by Longmuir for bacteria (16) and for liver cells (17) and by Baender and Kiesse (4) for mitochondria. Second, Bagavan and Eiler (5) and Ashley (3) found that *Tetrahymena pyriformis* GL

[4] By exponential increase in respiration rate R with time t, it is meant that $R_2 = R_1 e^{k(t_2 - t_1)}$ where k is constant in the time interval spanned by t_1 and t_2. In this interval the exponential rate constant k is the slope of the linear plot of log R versus t.

respiration was reduced more than 30% at an ambient oxygen concentration (1.4% by volume) almost 10 times greater than that used for hypoxic synchronization (0.15%). Third, Fig. 3 shows a sharp increase in *Tetrahymena* respiration rate just after the seventh hypoxic shock.

Hypoxic shock significantly reduces both respiration rate and intracellular ATP concentration, whereas hyperthermic shock does not significantly reduce respiration rate and reduces intracellular ATP only slightly. It therefore appears that hypoxia, but not hyperthermia, reverses progress toward division by reducing ATP concentration through reduction of the rate of oxidative phosphorylation. Blockage of *Tetrahymena* cell division by a 50% reduction of intracellular ATP concentration is consistent with Epel's finding (11) that mitosis of fertilized sea urchin eggs was blocked by a 50% reduction in ATP content.

Although hypoxic and hyperthermic shocks differ in their effect upon *Tetrahymena* ATP, it is possible that both of them may reverse progress toward division by the same *underlying* mechanism. Byfield and Scherbaum (7) have shown that hyperthermic shock reduces the rate of protein synthesis. Moner (20) found that hyperthermic shock reduced the rate of RNA synthesis and virtually eliminated the peak of pulse uridine-^{14}C incorporation into RNA, which he found to occur 30 min after the last of a series of synchronizing hyperthermic shocks.

Since ATP is required for amino acid activation and for synthesis of other ribonucleoside triphosphates (CTP, GTP, and UTP), hypoxic depression of intracellular ATP concentration may be expected to reduce the rates of both protein and RNA syntheses. If this is the case, both hypoxic and hyperthermic shocks cause a reduction of RNA and protein syntheses.

Byfield and Scherbaum (7) have shown that hyperthermic shock increases the rate of degradation (probably enzymatic) of pulse-labeled RNA. Such degradation of RNA is consistent with the concept (see references 12, 13, 19, 21, 22, 24, 30, 31) that at approximately 40 min after the end of a temperature shock sufficient mRNA (called "division mRNA") is synthesized to template specific protein(s) (called "division protein") which will trigger the ensuing division. If so, the division mRNA is present at the end of a 40 min period between temperature shocks. If the RNA were not degraded during a temperature shock, it

would be expected to template division protein as soon as the temperature shock ended, and the ensuing division could not be blocked by actinomycin D, an inhibitor of RNA synthesis. Actinomycin D, however, does block *Tetrahymena* division as much as 40 min after the final of a series of hyperthermic shocks (22). *If* division mRNA is synthesized between hypoxic shocks, then hypoxic shocks, which may be placed at least 55 min apart[5] and which, like temperature shocks, delay division by a period greater than their duration, probably permit a degradation of RNA

It is interesting that hypoxic shocks, which are carried out at a temperature (29°C) intermediate between hyperthermic shock (34°C) and hypothermic shock ($7\frac{1}{2}$°C) (33), require a period of application (40–45 min) intermediate between that of hyperthermic shock (20 min) and that of hypothermic shock (120 min) (33). This might be interpreted to indicate a temperature dependence of the rate of some reaction necessary for reversal of the cell's progression toward division. RNA degradation, shown by Byfield and Scherbaum (7) to be temperature dependent between 29° and 34°C, could be such a reaction. At 29°C under air, the cells synthesize ATP at a rate rapid enough to sustain RNA synthesis at a rate greater than the rate at which RNA is enzymatically hydrolyzed. At 29°C during hypoxia, the decreased intracellular ATP concentration may decrease RNA synthesis but not RNA hydrolysis, causing a net loss of RNA (including the postulated division mRNA).

Respiration after Synchronization

The finding that before the first synchronized division the respiration rate per cell was somewhat greater after hypoxic synchronization and much greater after hyperthermic synchronization than in exponential phase culture is consistent with the previous findings (25) that cell volume is somewhat greater before the first hypoxically synchro-

nized division and much greater before the first hyperthermically synchronized division than in exponential phase culture. In the first few minutes after the last hypoxic shock, respiration rate per cell and cell volume (25) are only slightly greater than in an unsynchronized culture.

The pattern of increase in total respiration with time of hypoxically and hyperthermically synchronized *Tetrahymena* cells differed from that of the synchronous *Tetrahymena pyriformis* cells studied by Zeuthen (32). This is to be expected since the relatively greater increase in cell number than in total respiration rate (Fig. 3) is indicative of a return by synchronized cells to a state of "balanced growth" in which respiration per cell is less. Although both synchronous cells (32) and synchronized cells (Fig. 3) display an abrupt acceleration of total respiration at the completion of fission, synchronous *Tetrahymena* cells exhibit a nearly linear increase in respiration rate with time between divisions, as opposed to the exponential increase in total respiration observed before and after hypoxically and hyperthermically synchronized division. With regard to the concepts of metabolic amplification and distortion presented in the Introduction, comparison of Fig. 3 with the data of Zeuthen (32) indicates that the pattern of respiration increase after *either* hypoxic or hyperthermic division synchronization is distorted from that of an unperturbed cell in exponential culture. In this case, had Zeuthen's data not existed, the alternate procedure approach would have led to the false impression that *Tetrahymena* respiration increases exponentially between divisions. On the other hand, the alternate procedure approach has indicated correctly that there is an acceleration of total respiration just after the completion of fission

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