JOURNAL OF BACTERIOLOGY, Aug. 1968, p. 330-337
Vol. 96, No. 2

Adenosine Triphosphate and Synchronous Mitosis in *Physarum polycephalum*

B. CHIN AND I. A. BERNSTEIN

Cellular Chemistry Laboratory, Departments of Dermatology and Industrial Health, and The Institute of Industrial Health, The University of Michigan, Ann Arbor, Michigan 48104

Received for publication 20 May 1968

Synchronous mitoses occur in *Physarum polycephalum* in the absence of cell division. Nucleoside and nucleotide profiles were prepared from synchronously growing *P. polycephalum* at intervals throughout the growth cycle. Comparison of these profiles demonstrates that the pool of adenosine triphosphate decreases from a high level at prophase to a minimum through mitosis and increases again in the postmitotic period. These events appear to coincide with changes in the pools of adenosine diphosphate and adenosine but not with that of adenosine monophosphate. This observed decrease in the pool of adenosine triphosphate during mitosis was confirmed by direct enzymatic assay. These results presumably reflect the energy demands of the cell during mitosis.

Physarum polycephalum is a very suitable biological model for studies on mitosis. This organism grows as a multinucleate plasmodium; the nuclei divide synchronously within a common cytoplasmic field in the absence of cell division (8). The immediate advantages provided by studying this organism are twofold: (i) mitotic events are amplified, simply because all the nuclei are doing the same thing at the same time, and (ii) mitotic events are not complicated by the process of cell division, as they usually are in the typical cell.

Fusion experiments with plasmodia at differing periods in their life cycles have demonstrated that mitotic synchrony is controlled through the cytoplasm (17). Some substance(s), presently unidentified, presumably pervades the cytoplasm to keep dividing nuclei in step or, at least, to initiate mitoses synchronously

Nucleoside and nucleotide pools from synchronously growing *P. polycephalum* were examined throughout the growth cycle. These compounds were selected because (i) they are probably easily transported throughout the cytoplasm, (ii) they may contribute to the energy pool for mitosis, (iii) they may serve as precursors for compounds required for mitosis, and (iv) they may play a role in initiating or maintaining synchrony.

MATERIALS AND METHODS

P. polycephalum was the gift of Odvaar Nygaard. The organism was grown on the soluble medium which was a modification of the one developed by Daniel and Rusch (5). The modified medium contained (g/liter): citric acid·H_2O, 2.2; $CaCl_2 \cdot 2H_2O$, 0.6; $MgSO_4 \cdot 7H_2O$, 0.6; $FeSO_4 \cdot 7H_2O$, 0.084; $MnCl_2 \cdot 4H_2O$, 0.084; $ZnSO_4 \cdot 7H_2O$, 0.034; Tryptone (Difco), 10.0; yeast extract (Difco), 1.5; KH_2PO_4, 2.0; dextrose, 10.0; Hemin (Nutritional Biochemicals Corp.), 0.005; final *p*H 5.0. Growth medium was always made and sterilized in three parts: basic medium, dextrose solution, and hemin solution. In turn, the basic medium was made in two portions: the first contained all components exclusive of Tryptone, yeast extract, and KH_2PO_4, at *p*H 3.5, and the second portion consisted of Tryptone, yeast extract, and KH_2PO_4 in solution. After mixing both portions, the *p*H of the basic medium was adjusted to 4.6. A final mixing of basic medium, dextrose, and hemin solutions produced the complete growth medium. Hemin solution was made as follows: 0.0063 g of hemin was dissolved in 20 ml of 1% NaOH, brought to *p*H 8.0 with H_3PO_4, diluted to 25 ml, and sterilized by membrane filtration. The complete medium contained 20 ml of hemin solution per liter.

The organism was maintained in submerged culture by serial transfer every 3 days into 40 ml of growth medium in a 250-ml Erlenmeyer flask. Submerged cultures were incubated at 21 C in a New Brunswick Psychrotherm model G-27 incubator-shaker (1-inch stroke diameter) at 165 rev/min.

Mass synchronized surface cultures were prepared by a modification of the method described by Nygaard, Guttes, and Rusch (14). Submerged cultures (3 days old) were harvested by allowing them to stand momentarily; the organism sank rapidly to the bottom of the flasks. The cultures were removed, along with a minimum of medium, with a pipette and allowed to run out of the pipette into a sterile flask. A thick slurry was produced. The slurry was picked up with a pipette and inoculated onto filter paper supported by glass

beads in 9-cm petri dishes. The diameter of each spot of inoculum was approximately 5/8 inch. The usual experiment consisted of 50 to 100 plates with 4 to 5 spots of inoculum per plate. After 3 hr, for fusion of the microplasmodia, fresh growth medium was added. Care was taken to adjust the level of medium to the surface of the filter paper and to remove air bubbles trapped beneath the paper. The first synchronized mitoses occurred 7 to 9 hr after addition of the medium; the interval between the first and second mitoses was 12 to 14 hr.

Preliminary experiments demonstrated that a few important points had to be observed for preparation of healthy, synchronized, surface cultures. Inoculum from submerged cultures must be young and should contain a minimum of slime. Centrifugation, as a means of concentrating the inoculum, could not be used. The duration of the fusion period was kept to the minimal time which was adequate.

The degree of synchrony was determined by measuring the mitotic index. Cultures were harvested at early prophase and were passed through a 26-gauge needle three times. Wet mounts were made from these crude homogenates and examined with a phase-contrast microscope. Nuclei with acentric, extremely crescent-shaped nucleoli on the verge of disappearing (early prophase) and nuclei whose nucleoli had disappeared were scored as having entered mitosis; nuclei with centrally located nucleoli were scored as not having entered mitosis. Systematic examination of 500 intact nuclei per mount revealed that the mitotic index was always in excess of 95%.

Acid-soluble extracts were prepared from cultures harvested at intervals throughout the growth cycle; the extraction procedure is presented in Fig. 1. The efficiency of extraction was followed by measuring absorbance at 260 and 275 mμ. Appreciable loss of ultraviolet-absorbing material occurred only when the pooled preparation was diluted with one volume of ice-cold ethyl alcohol. This step was necessary to precipitate viscous material which, if not removed, restricted columnar flow. However, absorption spectra of the ethyl alcohol precipitates, prepared after solution in 0.6 N $HClO_4$, were not indicative of the presence of nucleic acid materials but did show "end absorption." These fractions were subsequently discarded. The dry weight of the cells harvested for examination by column chromatography were in the range of 0.6 to 1.3 g.

Nucleoside and nucleotide profiles were prepared by ion-exchange chromatography (2, 3, 9). The effluent from these columns was monitored at 260 and 275 mμ with a Gilford recording spectrophotometer. Nucleosides were separated from nucleotides by passing the diluted acid-soluble extract through a column (1.8 cm diameter × 25 cm) of Dowex-1 formate at neutral *p*H. Nucleosides passed directly through, while nucleotides were retained. After washing the column with distilled water, nucleotides were separated by linear gradient elution with 1 liter of distilled water in the mixing flask and 1 liter of 1 M $HCOONH_4$ in the reservoir. When the reservoir had emptied, chromatography was extended with the addition of 1 liter of 2 M $HCOONH_4$ to the reservoir.

Nucleosides in the wash from the nucleotide column were concentrated by flash evaporation. They were subsequently separated by gradient elution on columns of Dowex-1 formate equilibrated with 0.02 M $HCOONH_4$ adjusted to *p*H 10.6 with ammonia (3). The column dimensions were 1.8 cm diameter × 25 cm; elution was started with 0.02 M $HCOONH_4$ adjusted to *p*H 10.6 with NH_3 in a 100-ml mixing flask; the reservoir contained 400 ml of 0.2 M $HCOONH_4$ adjusted to *p*H 10.6 with ammonia. When the reservoir had emptied, the mixing flask was disconnected, and elution was continued with 1,100 ml of 0.2 M $HCOONH_4$ adjusted to *p*H 10.6 with ammonia.

Preliminary identification of nucleotides and nucleosides was made by comparison of elution volumes and absorbancy ratios of 275 to 260 mμ with standard compounds (9).

More definitive identification was obtained by a comparison of absorption spectra (1), after removal of $HCOONH_4$, and by thin-layer chromatography. Pooled peaks were concentrated by flash evaporation. $HCOONH_4$ was removed by sublimation on a cold finger (cooled with liquid N_2) under high vacuum in a system including an oil-diffusion pump. Fractions which appeared to contain more than one nucleotide were chromatogrammed in 70% isopropanol on S and S 589 filter paper. The nucleotides were located on paper with a Mineralight model V-41 ultraviolet lamp, cut out, and eluted from the paper with water. Absorption spectra were obtained with a Beckman DB recording spectrophotometer at *p*H 1 (0.1 N HCl), *p*H 7 (0.02 M sodium phosphate), and *p*H 11 (0.002 M NaOH). Thin-layer chromatography was accomplished by using plates prepared with MN-Polygram cel 300/uv_{254} (Brinkman Instruments, Inc., Westbury, N.Y.). Solvent systems for nucleotides were saturated $(NH_4)_2SO_4$-1 M sodium acetate-isopropanol (80:18:2, v/v/v) and tertiary amyl alcohol-formic acid-water (30:20:10, v/v/v). Nucleosides and bases were separated by thin-layer chromatography with water. Isolated compounds were chromatogrammed with and against standards; all compounds were located on thin-layer plates with the Mineralight ultraviolet lamp.

ATP in acid-soluble extracts was also measured enzymatically for greater accuracy and specificity. One or, at most, two culture flasks were used to prepare inoculum for plates. Twenty-five plates were inoculated, each with one spot of inoculum. The cultures were examined microscopically as they approached the second mitosis to determine the time of early prophase (zero-time). This morphological point was defined as the period during which the nucleolus is extremely crescent-shaped and on the verge of disappearing. Preliminary experiments demonstrated that, while all cultures did not precisely enter the second division together, mitoses, once initiated in each culture, were essentially completed in 90 min; the reappearance of single nucleoli was considered the end point of mitosis. At each interval of 0, 15, 30, 60, 90, and 120 min after early prophase, 12 to 15 cultures were harvested. Acid-soluble extracts were prepared for enzymatic analysis (Fig. 1); the final volume of each sample was reduced to 5.0 ml by

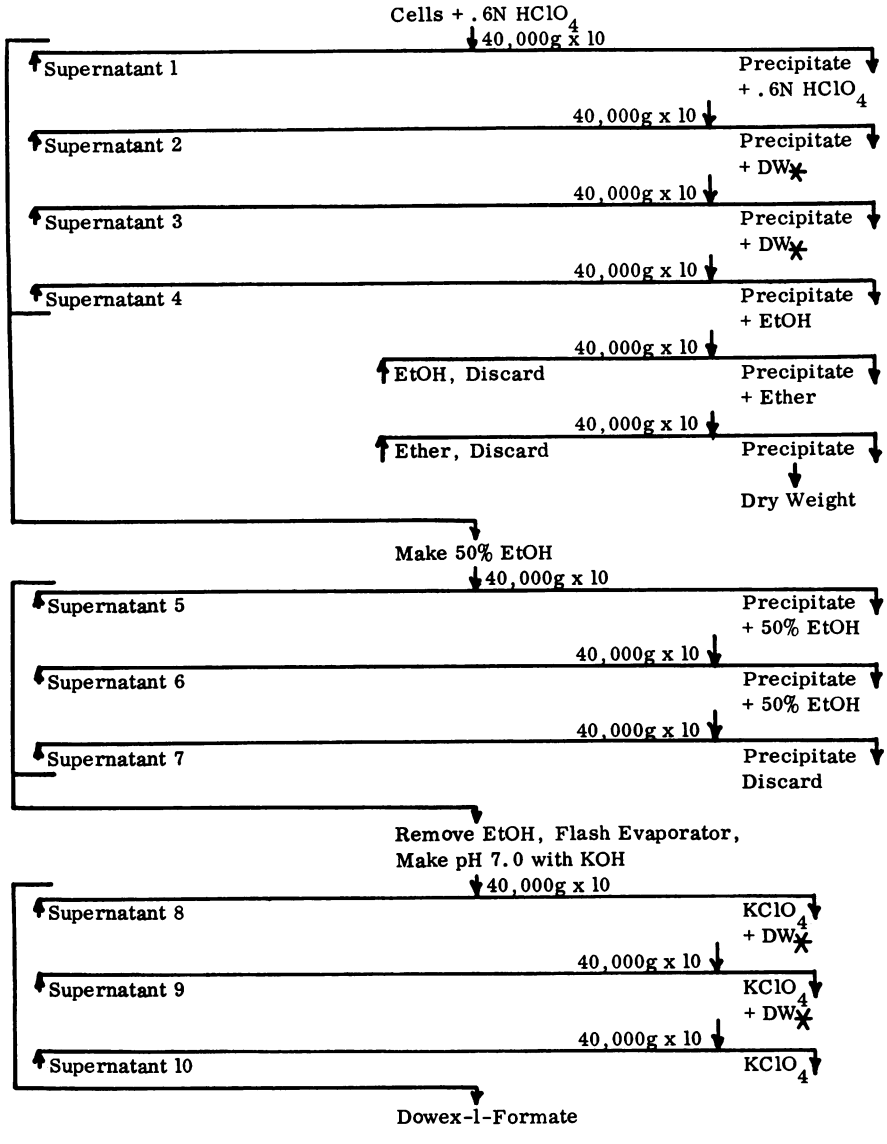

FIG. 1. *Flow sheet for preparation of acid-soluble extracts from P. polycephalum. Asterisk (*) = distilled water.*

flash evaporation. The dry weights of the cells in these samples were on the order of 130 to 250 mg.

Adenosine triphosphate (ATP) was assayed by coupling a gluconokinase, prepared from *Escherichia coli* in this laboratory by a modification of the method of Scott and Cohen (18), with commercial 6-phosphogluconate dehydrogenase (Boehringer). The gluconokinase did not react with uridine triphosphate or guanosine triphosphate at 10 times the concentration of ATP used in these assays. Cytidine triphosphate was utilized by the enzymatic preparation at one-tenth the rate of ATP utilization when cytidine triphosphate was present at 10 times the usual concentration of ATP.

The reaction mixture, incubated at 37 C, contained 1.0 ml of 0.1 M tris(hydroxymethyl)aminomethane buffer (*p*H 7.8), 0.3 ml of 0.01% ethylenediaminetetraacetic acid, 0.3 ml of 0.1 M $MgCl_2$, 0.3 ml of 0.004 M nicotinamide adenine dinucleotide phosphate, 0.1 ml of 0.05 M sodium gluconate, 0.1 ml of gluconokinase, 0.1 ml of 6-phosphogluconate dehydrogenase, 0.1 ml of ATP or sample; final volume, 3.0 ml. Enzymes were added in amounts to make the initial reaction rate dependent on ATP content up to 0.10 μmole. The reaction was measured on a Gilford recording spectrophotometer by following reduction of nicotinamide adenine dinucleotide phosphate at 340 mμ.

RESULTS

Nucleoside and nucleotide profiles developed at intervals throughout the growth cycle are presented in Fig. 2 and 3. The intervals examined were: D_1 (early prophase of the first mitotic division); D_1, 1 (1 hr after D_1); D_1, 4 (4 hr after D_1); D_1, 8; D_1, 12; and D_2 (early prophase of the second mitotic division). Elution positions and absorption ratios were taken as preliminary evidence for the identities of the compounds in the peaks (9). These compounds were subsequently identified by absorption spectra and thin-layer chromatography.

Usually, eight distinct peaks appeared on each nucleotide profile (Fig. 2). The first peak was absent at D_1, 8. Adenosine monophosphate, ATP, uridine monophosphate, uridine triphosphate, guanosine diphosphate, guanosine triphosphate, and urocanic acid were isolated and identified. The identities of the first two peaks and the peak preceding adenosine diphosphate are presently unknown; on the basis of ratios of 275 to 260 mμ, they do not appear to be uridine diphosphate (0.60), guanosine monophosphate (0.77), cytidine monophosphate (1.15), cytidine diphosphate (1.15), or cytidine triphosphate (1.15). The most striking change in these profiles is the rapid decrease in the size of the ATP pool during mitosis. This change occurs with a concomitant increase in the adenosine diphosphate pool but not the adenosine monophosphate pool.

Adenosine, adenosine diphosphate, and ATP fractions from the profiles were combined. After the volume and absorbancy were measured, the quantity of each peak was converted to micromoles per gram (dry weight) of cells by using the extinction coefficient for each compound. These results are presented in Table 1, which shows that the level of ATP falls from 7.08 to 2.28 μmoles/g during the first hour of mitosis. At the same time, the adenosine pool also decreases from 7.59 to 5.95 μmoles/g, while the adenosine diphosphate pool increases from 6.04 to 10.32 μmoles/g. Subsequently, during interphase, the ATP pool rises

TABLE 1. *Adenosine (AR), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) levels in Physarum polycephalum at intervals throughout the growth cycle. The intervals are D_1 (early prophase of the first mitotic division); D_1, 1 (1 hr after D_1); D_1, 4 (4 hr after D_1); D_1, 8; D_1, 12, and D_2*

Time (hr)	Dry wt	Absorbancy at 260 mμ			Dry wt (μmoles/g)		
		AR	ADP	ATP	AR	ADP	ATP
	g						
D_1	0.677	79.2	63.0	73.8	7.59	6.04	7.08
D_1, 1	0.675	62.0	107	23.8	5.95	10.3	2.28
D_1, 4	0.819	62.4	62.7	48.3	4.95	4.97	3.84
D_1, 8	0.808	81.2	67.6	55.6	6.52	4.39	4.47
D_1, 12	0.868	70.4	60.9	56.7	5.30	4.56	3.68
D_2	1.38	141	117	117	6.66	5.52	5.55

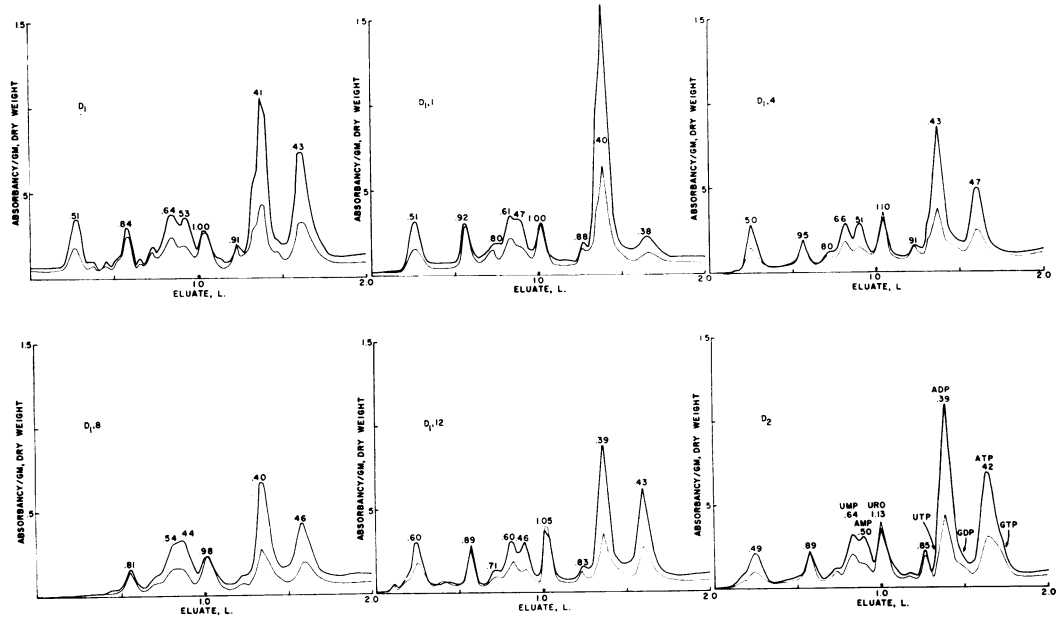

FIG. 2. *Nucleotide profiles of P. polycephalum prepared at intervals throughout the growth cycle. Heavy lines are recordings of 260-mμ absorbance; light lines are recordings of 275-mμ absorbance. Numbers over the peaks are ratios of 275 to 260 mμ.*

to a value of 5.55 μmoles/g at D_2. Adenosine monophosphate was not included in these calculations because any changes in this component, estimated from the profiles, appeared to be small. The error contributed by uridine triphosphate and guanosine triphosphate in ATP pools was similarly disregarded because their levels were low throughout the growth cycle.

The decrease in ATP during mitosis was confirmed by enzymatic analysis. This method provided the advantages of faster and more specific determinations on smaller samples. In turn, since each culture in a sample was examined microscopically, the mitotic times of samples were determined with greater precision. The results from this series of experiments are presented in Table 2. ATP falls from 6.28 μmoles/g at early prophase of the second division to a level below the limits of the assay system at 60 min after early prophase and rises to a level of 9.56 μmoles/g at 90 min after early prophase. This time, 90 min after early prophase, marks the morphological end point of the second mitosis, as single nucleoli are reappearing in nuclei. The level of ATP at D_2, as determined by enzymatic analysis (6.28 μmoles/g), agrees well with measurements from nucleotide columns (5.55 μmoles/g).

Cytidine, adenosine, uridine, and thymine were isolated and identified in fractions from nucleoside columns (Fig. 3). The first eluted peak always contained a flocculent precipitate and was subsequently discarded. The minor peak which follows adenosine has not been identified. This fraction does not contain guanosine; the 275/260 mμ ratio for guanosine at this *p*H is 0.85; guanosine, if it were present in these samples, would be eluted after uridine from these columns. Adenosine falls from a premitotic level of 7.59 to 5.95 μmoles/g during the first hour of mitosis in the first division (Table 1).

Figure 4 presents nucleoside and nucleotide profiles taken from a sample harvested 1 hr prior to D_2. The patterns are characteristic except for a new peak preceding adenosine. This component appears and disappears very rapidly just prior to mitosis. The identity of this compound and the role it may play in mitosis are currently under investigation. This compound has been isolated by ion exchange and gel-filtration chromatography. Absorption spectra of this substance contained one maximum at 275 mμ when they were developed at *p*H 1.0, 7.0, and 11.0; it does not appear to be one of the known nucleic acid derivatives (1, 20). The molecular weight of this material has been estimated by gel filtration to be in the range of 700 to 1,500. Our interest in this substance is prompted by the possibilities that it may be (i) a compound required for mitosis, (ii) a precursor of a compound required for mitosis, (iii) a degradation product of some reaction necessary for mitosis, or (iv) have no direct relationship with mitosis, but reflect some rhythmic process during synchronous growth in this organism.

TABLE 2. *Enzymatic analysis of ATP during the second synchronous mitosis in P. polycephalum*[a]

Time after D_2	Dry wt	Absorbancy[b]	ATP/0.1-ml sample	ATP/g (dry wt)
			μmole	*μmole*
0	159	0.008	0.020	6.28
15	215	0.010	0.025	5.82
30	203	0.007	0.018	4.43
60	152	0.001	0.003	0.99
90	131	0.010	0.025	9.56
120	256	0.012	0.030	5.86

[a] Sample size was 5.0 ml. The assay with ATP as standard is directly dependent on ATP to 0.10 μmole with a rate of 0.040 $\Delta A_{340} \times (2 \text{ min})^{-1} \times (0.10 \text{ μmole of ATP})^{-1}$.

[b] $A \times (2 \text{ min})^{-1} \times (0.1\text{-ml sample})$.

DISCUSSION

Mitosis has been extensively described as a highly ordered sequence of morphological steps (10). The genetic material, deoxyribonucleic acid (DNA), of *P. polycephalum* is replicated during the S period of the interphase preceding mitosis and not during mitosis per se (7, 14); this organism appears to have a very short G_1 period. The primary structure of the chromosome of the eucaryotic cell appears to consist of a thread, approximately 40 A in diameter, containing one DNA molecule in association with basic protein (11). However, the biochemical events involved in orienting and moving these structures through the complex and rigid pattern of mitosis remain to be described. This difficulty is related, in part, to the scarcity or inhomogeneity of experimental material, i.e., only a small fraction of a population of cells is in mitosis at any given time. This situation appears to have been corrected by the development of synchronous systems (4, 21).

Synchronous mitoses occur in *P. polycephalum* in the absence of cell division (8). This phenomenon seems to occur wherever nuclei exist in a common cytoplasmic field (10, 16) and presents another opportunity to study the complex interaction between nucleus and cytoplasm. The cytoplasm of *P. polycephalum* is not homogeneous, but contains the usual array of subcellular components (6, 12). In spite of this, the nuclei divide synchronously. Some substance or substances must pervade the cytoplasm to establish a mitotic environment around each nucleus and to keep the nuclei in step through division.

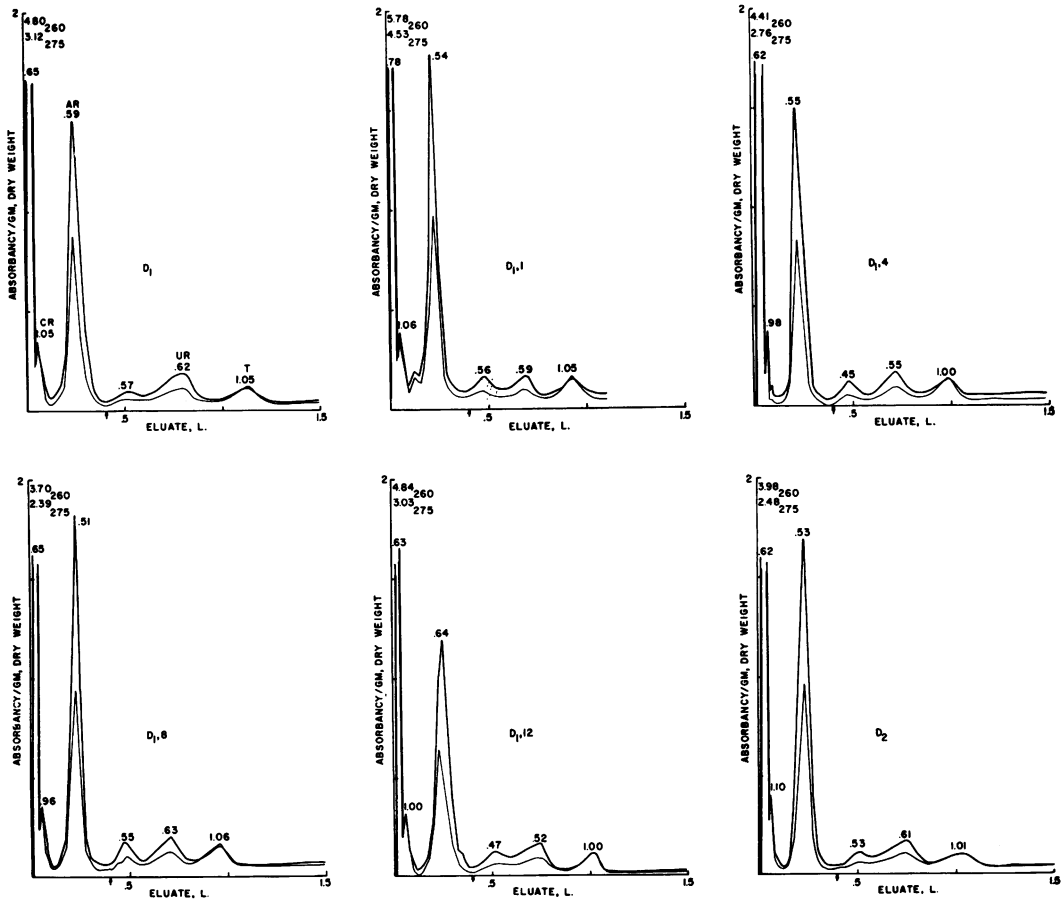

FIG. 3. *Nucleoside profiles of P. polycephalum prepared at intervals throughout the growth cycle. Heavy lines are recordings of 260-mμ absorbance; light lines are recordings of 275-mμ absorbance. Numbers over the peaks are 275/260 mμ ratios. Additional numbers over the first peak are the optical densities at the top of the peak; this fraction always exceeded the scale of these drawings.*

Although mitosis may be controlled by one substance which sets the pace for the process, the complexities involved imply that other substances play secondary, but still important, roles. One of these substances may be ATP. The experiments in this paper show that the pool of ATP decreases during mitosis. At this time, the rate of macromolecular synthesis is low: DNA is not synthesized (14), and ribonucleic acid synthesis (13) and protein (12) synthesis occur at much lower rates than in the premitotic period. In contrast, the ATP pool is high throughout interphase, when macromolecular synthesis proceeds at high rates, A possible correlation between the size of the ATP pool and macromolecular synthesis would have ATP increase when synthetic rates are low and decrease when synthetic rates are high. Our data do not follow this pattern. The depletion of ATP may reflect the energy requirements of mitosis.

In 1958, Swann (19) proposed, in a review of his experiments and those of other investigators on inhibitors and mitosis, that the energy for mitosis is stored in a reservoir during interphase and expended during mitosis. Sea urchin eggs require oxygen for growth. When they are placed under anaerobic conditions, the eggs are arrested in approaching mitosis, up to a critical point, for a period of time equal to the period of anaerobiosis. Exposure at or beyond the critical point does not impair the first mitosis following exposure; however, the second mitosis is delayed for a period equal to the duration of exposure. These results are duplicated if respiration is inhibited with carbon monoxide or dinitrophenol rather than anaerobic conditions. According to Swann,

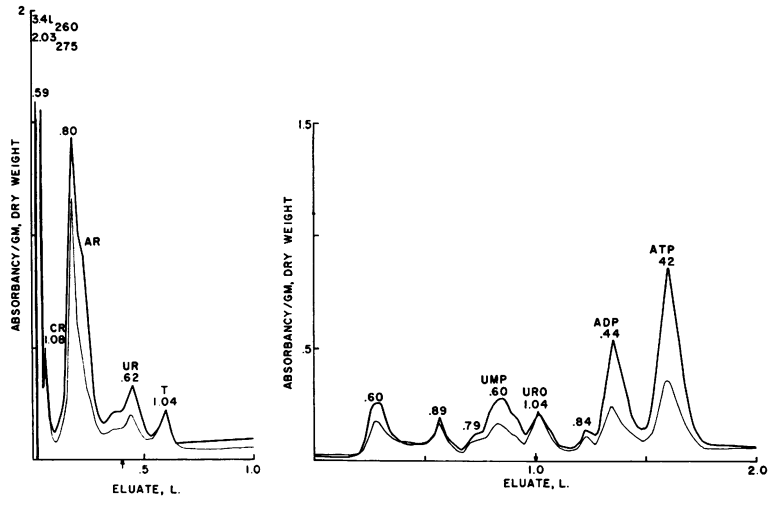

FIG. 4. *Nucleoside and nucleotide profiles of P. polycephalum prepared at 1 hr prior to the second synchronous nuclear division. Heavy lines are recordings of 260-mμ absorbance; light lines are recordings of 275-mμ absorbance. Numbers over the peaks are 275/260 mμ ratios. The additional numbers over the first peak from the nucleoside column are optical densities at the top of the peak; this fraction always exceeded the scale of these drawings.*

some tissue culture cells are not sensitive to anaerobic conditions or respiratory inhibitors. However, mitosis in these cells responds to the glycolytic inhibitors iodoacetate and fluoride in the same fashion in which sea urchin eggs respond to respiratory inhibitors. Swann postulated that respiration and glycolysis, depending upon the metabolism of the cell, contribute during interphase to an energy reservoir which is expended during mitosis. Since the energy reservoir would be full at a critical point prior to mitosis, the loss of energy resulting from inhibition of respiration and glycolysis at and beyond this critical point would not delay the first mitosis following inhibition. Although ATP did not appear likely to be the ultimate energy source, he proposed that ATP may represent the currency by which the reservoir is spent. His hypothesis predicts that the turnover rate of ATP would be high during mitosis. The experiments in this paper support his prediction, in part, by demonstrating that the rate of ATP utilization during mitosis exceeds the rate of synthesis.

ATP content has also been measured in synchronously growing *Tetrahymena pyriformis* (15) with results similar to those presented here. The ATP pool rises to a maximum prior to cell division, decreases before cytokinesis begins, and continues to decrease through and beyond cell division. Whether these fluctuations in *T. pyriformis* reflect the demands of mitosis or cytokinesis, or both processes together, is difficult to determine, since mitosis and cell division in this organism appear to be contiguous, if not overlapping, processes.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service training grant 2 TO1 AM 05268 from the National Institute of Arthritis and Metabolic Diseases.

LITERATURE CITED

1. Beaven, G. H., E. R. Holiday, and E. A. Johnson. 1955. Optical properties of nucleic acids and their components. *In* E. Chargaff and J. N. Davidson (ed.), The nucleic acids. Academic Press, Inc., New York.
2. Bernstein, I. A., and P. Foster, 1957. The metabolism of the nucleic acids in the skin of young rats. J. Invest. Dermatol. **29**:415–422.
3. Bernstein, I. A., and D. Sweet. 1958. Biosynthesis of deoxyribose in intact *Escherichia coli*. J. Biol. Chem. **233**:1194–1198.
4. Cameron, J. L., and M. Padilla (ed.). 1966. Cell synchrony: studies in biosynthetic regulation. Academic Press, Inc., New York.
5. Daniel, J. W., and H. P. Rusch. 1961. The pure culture of *Physarum polycephalum* on a partially defined soluble medium. J. Gen. Microbiol. **25**:47–59.
6. Guttes, D., S. Guttes, and H. P. Rusch. 1961. Morphological observations on growth and differentiation of *Physarum polycephalum* grown in pure culture. Develop. Biol. **3**:588–614.
7. Howard, A., and S. R. Pelc. 1951. Nuclear incorporation of P^{32} as demonstrated by autoradiographs. Exptl. Cell Res. **2**:178–187.

8. Howard, F. L. 1932. Nuclear division in plasmodium of *Physarum*. Ann. Botany (London) **46**:461–477.
9. Hurlbert, R. B., H. Schmitz, A. F. Brumm, and V. R. Potter. 1954. Nucleotide metabolism. II. Chromatographic separation of acid-soluble nucleotides. J. Biol. Chem. **209**:23–39.
10. Mazia, D. 1961. Mitosis and the physiology of cell division. *In* J. Brachet and A. E. Mirsky (ed.), The cell, vol. 3. Academic Press, Inc., New York.
11. Mazia, D. 1966. Biochemical aspects of mitosis. *In* The cell nucleus—metabolism and radiosensitivity. Taylor and Francis Ltd., London.
12. Mittermayer, C., R. Braun, T. G. Chayka, and H. P. Rusch. 1966. Polysome patterns and protein synthesis during the mitotic cycle of *Rhysarum polycephalum*. Nature **210**:1133–1137.
13. Mittermayer, C. R., R. Braun, and H. P. Rusch. 1964. RNA synthesis in the mitotic cycle of *Physarum polycephalum*. Biochim. Biophys. Acta **91**:399–405.
14. Nygaard, O., S. Guttes, and H. P. Rusch. 1960. Nucleic acid metabolism in a slime mold with synchronous mitosis. Biochim. Biophys. Acta **38**:298–306.
15. Plesner, P. 1964. Nucleotide metabolism during synchronized cell division in *Tetrahymena pyriformis*. *In* E. Zeuthen (ed.), Synchrony in cell division and growth. Interscience Publishers, Inc., New York.
16. Rosenberger, R. F., and M. Kessel. 1967. Synchrony of nuclear replication in individual hyphae of *Aspergillus nidulans*. J. Bacteriol. **95**:1464–1469.
17. Rusch, H. P., W. Sachsenmaier, K. Behrens, and V. Gruter. 1966. Synchronization of mitosis by the fusion of the plasmodia of *Physarum polycephalum*. J. Cell Biol. **31**:204–209.
18. Scott, D. B. M., and S. S. Cohen. 1953. The oxidative pathway of carbohydrate metabolism in *Escherichia coli*. I. The isolation and properties of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Biochem. J. **55**:23–33.
19. Swann, M. M. 1957. The control of cell division: a review. I. General mechanisms. Cancer Res. **17**:727–757.
20. Venkstern, T. V., and A. A. Baev. 1965. Absorption spectra of minor bases. Plenum Publishing Corp., New York.
21. Zeuthen, E. (ed.). 1964. Synchrony in cell division and growth. Interscience Publishers, Inc., New York.