Effect of Adenosine Monophosphate on Intermediate Metabolism and Ribonucleic Acid Synthesis in *Tetrahymena*

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SUMMARY

*Tetrahymena* were grown with shaking for 17 hours in the presence and absence of AMP. Under these conditions, glycogen content increased but there was a marked inhibition of glyoxylate oxidation and of the incorporation of label from acetate into glycogen during a 1-hour incubation. AMP treatment also caused an increase in the rate of incorporation of [3H]uracil into RNA which was maximal about 2 hours after the start of growth. Experiments were performed in which cells from log and transition phase cultures were incubated with acetate, pyruvate, and octanoate, but with only one substrate labeled in any one flask. Measurements were made of the amount of label incorporated from each labeled substrate into glycogen, lipids, CO₂, and glutamate, and the results were analyzed according to a previously established model for the structure of metabolism in *Tetrahymena*. It was found that the model, which utilizes three pools of acetyl-CoA, permitted a quantitative fit to the data. Analysis of the data showed that AMP treatment reduced the rate of the Krebs cycle, the rate of gluconeogenesis, and the rate of pyruvate utilization in both log and transition phase cells from these labeled substrates. Incorporation of label from acetate into glutamate, however, was increased by AMP treatment in log cells and decreased in transition phase cells. AMP treatment markedly decreased the fraction of label entering into gluconeogenesis from the peroxisomal pool of acetyl-CoA. There was no change in the ATP:ADP ratio in log cells treated with AMP, and a slight decrease in the sum ATP + ADP. In transition phase cells, however, the ATP:ADP ratio decreased and the sum of ATP + ADP also increased compared to control cells. Since many of the changes in intracellular flux patterns occurred in both log and transitional cells, changes in enzyme levels or control mechanisms other than the ATP:ADP ratios must be invoked to explain the effects of AMP on metabolism.

It was recently reported that several adenine nucleotides and in particular 5'-AMP brought about an increase in the glycogen content of *Tetrahymena* (1). This was thought to result from an increase in gluconeogenesis, with the carbon probably coming from some unidentified components of the proteose-peptone medium. At about the same time, it was observed that the intraperitoneal injection of adenosine into the rat caused a remarkable increase in the turnover of glycogen in the liver and of lipid in the fat pad (2). Since one might expect that changes in adenine nucleotide levels would alter the flow of metabolites at many steps in intermediate metabolism, it was of interest to measure intracellular flux rates in *Tetrahymena* treated with AMP and compare the pattern of flow to that observed in control cells. In a preceding paper (3) we have developed a model for the flow of acetyl-CoA between the mitochondrial and peroxisomal compartments and a methodology for measuring the amounts of each of the substrates (acetate, pyruvate, and octanoate) utilized for gluconeogenesis, lipogenesis, and oxidation via the Krebs tricarboxylic acid cycle. In this paper we present a quantitative analysis of the effect of AMP on the flow of labeled acetyl-CoA along these pathways in both log and transition phase cells. To provide some insight into the parameters which may be responsible for the observed changes in flux rates, we have also measured the effect of AMP treatment on ATP and ADP content, on the rate of RNA synthesis, and on the time course for development of the previously reported (1) inhibition of glyoxylate oxidation.

MATERIALS AND METHODS

Cells—*Tetrahymena pyriformis*, strain HSM, were grown axenically in a medium containing 1% proteose peptone (Difco) and 0.05% concentrated liver extract in 0.02 M potassium phosphate buffer adjusted to pH 6.5 with NaOH. All stock cultures were grown in a gyratory water bath in 500-ml Erlenmeyer flasks containing 100 ml of medium at 26°. Cells were counted with a Coulter Counter. Growth was expressed as the ratio Nf:Ni, where Nf is the cell density at the beginning of the overnight incubation and Ni is the final cell density.

Incorporation of Label—Measurements were done exactly as described by Raugi et al. (3). It was established that incorporation of label into lipids, CO₂, and glycogen was linear with time after an initial lag of 5 to 15 min for each labeled substrate except that, as in control cells (3), incorporation of label from octanoate into CO₂ in log cells was not linear until after a long lag period.

Measurement of ATP and ADP Levels—Cells were harvested and assayed for ATP and ADP as described by Liang et al. (4).
Measurement of RNA and RNA Synthesis—Total RNA was measured by the method of Schmidt and Tannhauser (5) as modified by Blum and Padilla (6). RNA synthesis was measured by incubating 10 ml of cells from an unshaken culture with 2 μCi of [3H]uracil in a 300-ml Erlenmeyer flask with shaking for 30 min at 26°C. At the end of this incubation, aliquots were removed and precipitated in 10% trichloroacetic acid; the pellet was washed twice with 5% trichloroacetic acid and the final pellet was dissolved in 0.5 ml of hyamine and counted in 15 ml of Counting Solution A (3).

Materials—Isotopically labeled substrates and 5'-AMP were purchased from Amersham-Searle and Sigma, respectively. Firefly extract and pyruvate kinase were purchased from Worthington. AMP was dissolved in distilled water, the pH adjusted to about 6.5, and sterilized by passage through ultrafine sintered glass filters.

RESULTS

Blum (1) demonstrated that one of the major effects of AMP on the metabolism of *Tetrahymena* was inhibition of the oxidation of glyoxylate. This was shown not to be due to an inhibition of glyoxylate oxidase, a peroxisome-associated enzyme (7), but probably to reflect an inhibition of malate synthetase or of malate transfer from the peroxisomes to the mitochondria. Fig. 1 shows the results of an experiment in which cells were grown with and without AMP and at various times samples were taken and the rate of oxidation of [2-14C]glyoxylate was assayed during a 1-hour incubation. Initially 5'-AMP stimulated oxidation of glyoxylate to a small extent, but over a period of 6 hours the rate of oxidation declined to <30% of the value at zero time for both the control and AMP-treated cultures. At this point both cultures were in the logarithmic phase of growth and the AMP treated cells had a reproducibly smaller capacity to oxidize [2-14C]glyoxylate than control cells. Subsequently the rate of oxidation of this substrate began increasing in control cultures, but did not increase or continued to decline in AMP-treated cultures. The cultures used for analysis of metabolic flux patterns to be described below were all exposed to AMP for about 17 hours, and thus had very low glyoxylate-oxidizing capacity compared to their controls.

Effect of AMP on RNA Synthesis Rate—In order to determine whether the effect of AMP was restricted primarily to transient modifications of ongoing pathways or whether it was also likely that changes in enzyme levels were occurring, total RNA content and the rate of synthesis of RNA were measured at various times during growth of control and AMP-treated cultures (Fig. 2). In control cultures RNA content increased slightly at first and then declined to 75% of the original level. The RNA content of AMP treated cells did not decline but instead increased slightly. Although the differences in RNA content between control and AMP-treated cells were not marked, there was a striking difference in the rate of incorporation of [3H]uracil into RNA between treated and untreated cultures. It has elsewhere been shown that the pool of uracil or uracil-containing nucleotides in *Tetrahymena* was not expanded in response to treatment of the cells with actinomycin D or glucose (8) and only slightly expanded in response to increasing growth temperature from 28°C to 34°C (9). In *Tetrahymena*, furthermore, there is no interconversion between adenine compounds and uridine compounds (10). It therefore seems unlikely that exposure of cells to AMP caused an appreciable change in uracil pool size, and it will be assumed that the rate of incorporation of label from [3H]uracil reflects the rate of synthesis of RNA. Within 2 hours after addition of AMP,

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**Fig. 1.** Effect of 5'-AMP on growth and oxidation of [2-14C]glyoxylate in *Tetrahymena*. At *t* = 0, cells were added to 500-ml Erlenmeyer flasks containing 40 ml of proteose-peptone medium and 3.6 ml of water or 5'-AMP and incubated at 26°C with shaking. At the indicated times, duplicate 3-ml aliquots were removed from both flasks and incubated with 6.2 mM glyoxylate containing [2-14C]glyoxylate (25 dpm per nmole) for 1 hour, and 14CO2 production was measured. Units of glyoxylate oxidation are nmol of [2-14C]glyoxylate oxidized/10⁶ cells per hour.

**Fig. 2.** Effect of 5'-AMP on total RNA content and rate of RNA synthesis in *Tetrahymena*. At *t* = 0, cells were added to 500-ml Erlenmeyer flasks containing 10 ml of proteose-peptone medium and 2.5 ml of water of 5'-AMP (final concentration 3.6 mM) and incubated at 26°C without shaking. At the indicated times, aliquots were removed for assay of total RNA content and of [3H]uracil incorporation as described in under "Materials and Methods." N₀ = 248,000 cells per ml. Units of uracil incorporation are pmol of [3H]uracil incorporation/30 min/10⁶ cells.
there was a 7-fold increase in the rate of uracil incorporation into RNA. With time the difference between AMP treated and control cultures declined, but at 17 hours the AMP-treated cultures still incorporated uracil into their RNA about 1.6 times as fast as control cells. Thus from both the studies on the temporal development of inhibition of glyoxylate oxidation capacity and of rate of uracil incorporation into RNA it appears likely that changes in enzyme levels may occur in *Tetrahymena* in response to addition of AMP to the medium. In what follows, we examine the flux of metabolites and the ATP and ADP levels in cells exposed to AMP for about 17 hours. Further work would be required to analyze any changes in enzyme levels that may exist at this time.

**Effect of AMP on Oxidation and Incorporation into Glycogen of Glucose, Glyoxylate, and Acetate**—Table I shows the results of experiments in which 14CO₂ production and the incorporation of label into glycogen was measured for [1-14C]acetate, [1-14C]glucose, and [2-14C]glyoxylate when these substrates were added individually to cultures that had been grown for 17 hours with or without AMP. There were no changes in the rates of oxidation of acetate or glucose to CO₂ but, as described above, oxidation of glyoxylate was strongly inhibited by treatment with AMP. AMP caused a strong inhibition of label incorporation into glycogen from both glyoxylate and acetate, but enhanced the incorporation of glucose into glycogen. It therefore appears that the inhibition of glyconeogenesis from acetate and glyoxylate occurs prior to the formation of glucose 6-phosphate and its incorporation into glycogen.

**Effect of AMP on Intra-Cellular Distribution of Acetyl-CoA**—Since the above described experiments using individual labeled substrates indicated a marked inhibition of glyconeogenesis, it was of interest to attempt to quantitate the flow of acetyl groups along the major pathways of intermediate metabolism, as described by Raugi et al. (3). For this purpose, cells in the log and transition phases of growth were incubated for 1 hour with a mixture of 6.2 mM acetate, 6.2 mM pyruvate, and 1.5 mM octanoate, with only one of these substrate labeled at a time such a way that [1-14C]acetyl-CoA was the initial metabolite formed. Table II shows the results obtained from control cultures and cultures grown for about 17 hours in the presence of 3.0 mM AMP. Data for the control cultures formed part of the data used previously to establish the existence of three compartments of acetyl-CoA in *Tetrahymena* (3).

In both log and transition phase cultures, AMP reduced the

| TABLE I |
|---|
| **Effect of \( \delta' \)-AMP on oxidation and incorporation into glycogen of glucose, glyoxylate, and acetate** |
| Cells were grown in a total volume of 43 ml in 500-ml Erlenmeyer flasks in the presence or absence of 3.0 mM AMP at 26° with shaking for 17 hours. Cultures were harvested in transition phase. Aliquots were incubated with either 6.2 mM [1-14C]acetate (75 dpm per nmole), or 6.2 mM [2-14C]glyoxylate (23 dpm per nmole), or 6.2 mM [1-14C]glucose (25 dpm per nmole), and production of 14CO₂ and incorporation of label into glycogen were measured during a 1-hour incubation. |

| Labeled substrate | Experiment | Amount incorporated into |
|---|---|---|
| | | CO₂ | Glycogen |
| | | Control | AMP | Control | AMP |
| | | nmoles/10⁶ cells·hr |
| [1-14C]Acetate | A | 701 | 678 | 23.0 | 6.0 |
| | B | 809 | 819 | 40.5 | 10.0 |
| [1-14C]Glucose | A | 55.5 | 57.8 | 774 | 1100 |
| | B | 74.2 | 90.5 | 756 | 1140 |
| [2-14C]Glyoxylate | A | 36.4 | 6.6 | 12.0 | 7.0 |
| | B | 43.0 | 18.0 | 28.5 | 10.0 |

**TABLE II**

| Substrate and cultures | CO₂ | Lipids | Glycogen | Glutamate |
|---|---|---|---|---|
| Log cultures | | | | |
| [1-14C]Acetate | 384.9 ± 52.3⁻ | 143.3 ± 23.3⁻ | 3.8 ± 1.8⁻ | 6.2 ± 1.0⁻ |
| | (409.9 ± 40.6) | (158.9 ± 23.6) | (14.9 ± 1.2) | (4.4 ± 0.8) |
| [2-14C]Pyruvate | 45.3 ± 5.4⁻ | 1.9 ± 0.7 | 3.5 ± 2.3 | <0.5 |
| | (65.2 ± 6.3) | (2.1 ± 0.9) | (4.4 ± 1.3) | (<0.5) |
| [1-14C]Octanoate | 3.7 ± 0.9 | 31.0 ± 2.8 | 0.21 ± 0.00⁷ | <0.1 |
| | (7.4 ± 1.0) | (33.7 ± 5.0) | (0.48 ± 0.04) | (<0.1) |
| Transition cultures | | | | |
| [1-14C]Acetate | 406.2 ± 10.2⁷ | 154.3 ± 17.2 | 5.3 ± 1.4⁷ | 4.5 ± 0.8⁷ |
| | (510.7 ± 17.2) | (141.4 ± 11.4) | (15.2 ± 2.1) | (7.7 ± 1.6) |
| [2-14C]Pyruvate | 47.3 ± 2.1⁷ | 1.8 ± 0.6 | 3.6 ± 1.0⁷ | <0.5 |
| | (71.9 ± 4.8) | (1.9 ± 0.1) | (0.0 ± 0.9) | (<0.1) |
| [1-14C]Octanoate | 3.8 ± 2.8 | 34.0 ± 2.7 | 0.26 ± 0.12 | <0.1 |
| | (5.6 ± 2.2) | (31.5 ± 2.6) | (0.45 ± 0.14) |

* p < 0.05 by the Student t test.
FIG. 3. Effect of AMP on the flux of metabolism in Tetra-
hymena. The average parameter values listed in Table IV are
used to compile the scheme and are shown in parentheses below
the corresponding control values. An asterisk means p < 0.05
by the Student t test for paired data. A, log phase cells; B,
transition phase cells.

amount of label appearing in CO₂ and glycogen from all three of
the substrates used, but to differing extents. In log cells, for
example, acetate oxidation was reduced 20% while incorporation
of label from acetate into glycogen and lipids were reduced 65
and 10%, respectively, whereas incorporation into glutamate
was increased 40%. There was no change in incorporation of
label from pyruvate or octanoate into lipids. Similar results
were obtained for transition phase cultures except that the in-
corporation of label from acetate into glutamate was enhanced
in log phase cultures and reduced in transition phase cultures.

These results do not of themselves indicate the kinds of changes
occurring in the various pathways of intermediate metabolism,
but suggest that a number of changes in the distribution of
carbon must be occurring in response to AMP treatment.

Estimation of Flux Rates of Acetyl-CoA in AMP-treated Cells
—Because the metabolic effects of AMP are so diverse and ap-
parently complex, we attempted to fit each of the experiments
which have been averaged together in Table II according to the
metabolic scheme developed earlier and shown in Fig. 3. It was
found that this scheme gives a quantitative fit to the data (Table
III). The closeness of the fit obtained for this particular experi-
ment is in no way unrepresentative of the fits obtained to the
other eight experiments in which AMP was used. The average
values of the parameters of the model are listed in Table IV and
the flux along each pathway of the metabolic model is shown in
Fig. 3.

In log cells there was no significant change in α, the fraction
of acetate entering Pool III (the inner mitochondrial pool of
acetyl-CoA) or of β, the fraction of octanoate utilized via β
oxidation. There was a marked decrease, however, in β, the
fraction of octanoate β oxidized in Pool III, and a doubling of γ,
the fraction of glycogen originating from Pool I (the outer mito-
chondrial pool). Although the distribution of acetate between
the peroxisomes and the mitochondria was not changed by AMP,
the amount of acetate utilized was decreased in both the peroxiso-
al and mitochondrial pools (Pools II and III, respectively).
There was a small but significant decrease in total octanoate
utilization (V₂). The fraction of octanoate β oxidized (ε) was
essentially unchanged but AMP treatment caused a shift in the
intracellular location where β oxidation occurred; in AMP-
treated cells over twice as much octanoate was oxidized in the
peroxisomes as in the mitochondria, whereas in control cells
more octanoate was oxidized in the mitochondria than in the
peroxisomes.

Total pyruvate oxidation (V₃) was also decreased by AMP
 treatment. The decrease in V₃ was accompanied by a decrease
in V₁₄, the flow of acetyl-CoA from the outer to the inner mito-
chondrial pool of acetyl-CoA.

A particularly interesting finding vis-à-vis the above mentioned
reduction in glyoxylate oxidation capacity was the increase in γ,
the fraction of glycogen derived from Pool I (the outer mito-
chondrial pool) and the decrease in V₄, the total rate of glyco-
neogenesis. In the fact of the large reduction in V₄ the effect of
increasing γ was to maintain the mitochondrial contribution to
glyconeogenesis nearly constant compared to the large reduction
of the peroxisomal contribution to glyconeogenesis.

| Substrate        | Amount incorporated into |
|------------------|-------------------------|
|                  | CO₂ | Lipids | Glycogen | Glutamate |
| [1-¹⁴C]Acetate   |     |       |          |           |
| Observed         | 394.2 | 130.6 | 3.6 | 4.5 |
| Calculated       | 394.2 | 130.5 | 3.6 | 4.6 |
| [2-¹⁴C]Pyruvate  |     |       |          |           |
| Observed         | 44.9 | 1.7  | 2.3 | <0.5 |
| Calculated       | 44.9 | 1.7  | 2.3 | 0.5  |
| [1-¹⁴C]Octanoate |     |       |          |           |
| Observed         | 1.6  | 33.6 | 0.14 | <0.15 |
| Calculated       | 1.6  | 33.7 | 0.14 | 0.02  |
The calculated fluxes for four transition phase experiments and five log phase experiments were averaged. $\alpha$, $\beta$, $\gamma$, and $\epsilon$ are unitless. Other values are in nanomoles per $10^6$ cells per hour.

| Symbol | Description | Log Cultures | Transition Cultures |
|--------|-------------|--------------|---------------------|
|        |             | Control | AMP-treated | Control | AMP-treated |
| $\alpha$ | Fraction of acetate + pool III | 0.730±0.022 | 0.728±0.021 | 0.766±0.011 | 0.715±0.017* |
| $\beta$ | Fraction of octanoate $\beta$ oxidized + pool III | 0.60±0.06 | 0.312±0.015* | 0.54±0.13 | 0.29±0.14 |
| $\gamma$ | Fraction of glycogen from pool I | 0.22±0.05 | 0.47±0.09* | 0.28±0.02 | 0.43±0.04* |
| $\epsilon$ | Fraction of octanoate + $\beta$ oxidation | 0.30±0.05 | 0.39±0.15 | 0.28±0.05 | 0.31±0.09 |
| $V_1$  | CO$_2$ production | 542.4±42.3 | 441.1±51.8* | 588.4±21.8 | 455.2±14.5* |
| $V_2$  | Glutamate production | 5.0±0.5 | 6.7±1.6* | 8.6±1.2 | 5.1±0.8* |
| $V_3$  | Glycogen production | 19.8±1.0 | 7.5±3.6* | 21.6±2.8 | 9.2±2.4* |
| $V_5$  | Octanoate utilization | 41.7±2.4 | 39.0±2.6* | 37.9±4.9 | 37.9±5.5 |
| $V_6$  | Acetate utilization | 648.2±57.6 | 545.4±70.3* | 675.0±24.7 | 568.2±29.5* |
| $V_7$  | Pyruvate utilization | 72.5±3.2 | 51.2±5.3* | 80.8±5.1 | 53.6±3.2* |
| $V_9$  | Flow from pool I + pool II | 1.8±0.9 | 1.6±0.6 | 1.9±1.8 | 3.9±1.3 |
| $V_{10}$ | Flow from pool II + pool I | 2.4±1.0 | 2.1±0.8 | 2.2±0.1 | 2.2±0.7 |
| $V_{11}$ | Flow from pool III + pool I | 1.6±0.9 | 2.6±1.2 | 3.0±0.0 | 2.5±1.7 |
| $V_{14}$ | Flow from pool I + pool III | 68.8±6.1 | 49.6±5.3* | 77.3±5.6 | 53.4±2.5* |
| $\alpha V_6$ | Acetate + pool III | 472.9±40.8 | 397.3±51.9* | 517.1±15.7 | 405.7±14.6* |
| $(1-\alpha)V_6$ | Acetate + pool II | 175.3±24.4 | 148.1±23.1* | 157.9±11.9 | 162.6±17.0 |
| $\epsilon \beta V_5$ | Octanoate + pool III | 7.5±1.7 | 3.7±1.0* | 5.7±2.1 | 3.7±2.9 |
| $\epsilon (1-\epsilon)V_5$ | Octanoate + pool II | 5.0±0.1 | 10.0±2.2 | 5.0±2.1 | 8.1±2.5 |
| $(2-\epsilon)V_5$ | Direct incorporation of octanoate into lipids | 29.2±3.4 | 21.4±6.0 | 27.3±2.7 | 26.1±1.9 |
| $V_9-(1-\epsilon)V_5$ | Lipids from pool II | 165.6±24.2 | 154.9±38.8 | 147.7±12.7 | 163.7±10.4* |
| $\gamma V_3$ | Glycogen from pool I | 4.4±1.4 | 3.7±2.8 | 6.1±0.9 | 3.9±1.0* |
| $(1-\gamma)V_3$ | Glycogen from pool II | 15.3±1.3 | 3.8±1.8* | 15.5±2.1 | 5.9±0.8* |

* $p < 0.05$ by t test
adequate oxygenation even at the high cell densities of the transition phase cultures. Treatments with AMP, however, discloses several significant differences between log and transition phase cells. AMP treatments did not change α in log cells, but caused a significant decrease in α in transition cells. Thus AMP decreases the fraction of acetate utilized in the mitochondria relative to the peroxisomes in transition cells. Although practically the same amount of acetate entered the inner mitochondrial pool in both log and transition AMP-treated cells, more label from the acetate appeared in glutamate in log cells than in transition cells. Indeed, AMP treatments increased the rate of operation of the Krebs cycle (as indicated by Vl) to essentially the same value. In log cultures VS was decreased whereas Vg was increased. The model of metabolic scheme of Connett and Blum (11) peroxisomal isocitrate lyase splits isocitrate derived from the mitochondrial Krebs cycle into succinate, which returns to the mitochondria, and glyoxylate. Peroxisomal acetyl-CoA, derived primarily from acetate and secondarily from octanoate via peroxisomal β oxidation (12), condenses with glyoxylate via the action of malate synthetase. The peroxisomal malate so formed is either oxidized to oxaloacetate which can then enter the glyoxenogenic pathway via the cytosol phosphoenolpyruvate carboxykinase (11) or may re-enter the mitochondria where, presumably, it is oxidized in the Krebs cycle. The observation that 17 hours exposure to AMP strongly reduces glyoxylate oxidation and the incorporation of label from acetate into glyoxen from the peroxisomal compartment is consistent with the suggestion (1) that AMP treatment causes inhibition of malate synthetase or the transfer of malate from the peroxisomal compartment. In view of the change in RNA content and in the rate of synthesis of RNA in cells treated with AMP, it is possible that the reduction of carbon flux through the malate synthetase step may reflect a decrease in the amount of enzyme present or an inhibition of the activity of this enzyme. Although the analysis of intracellular flux rates as used in the present work cannot resolve this question, it does suggest that studies on factors controlling malate synthetase may be rewarding.

Oxidation of each substrate in the inner mitochondrial compartment was decreased in both log and transition cultures by AMP treatments. The decrease in rate of operation of the Krebs cycle could occur for a number of reasons, e.g., inhibition of citrate synthetase. An increased ATP:ADP ratio, as occurs in AMP-treated transition cells, is thought to inhibit the Krebs cycle, perhaps via inhibition of the citrate synthetase (13). One of the remarkable features of the present data, however, is that in AMP-treated log cells there was no change in ATP:ADP ratio and a small decrease in the sum ATP + ADP. Thus one cannot appeal to changes in ATP:ADP ratio to explain any of the changes which occurred in both log and transition cells in response to AMP treatment. The reason for the decrease in the rate of oxidation via the tricarboxylic acid cycle must remain a subject for further research.

It is noteworthy that although oxidative metabolism via the Krebs cycle decreased in both log and transition cells, the effect of AMP treatment on glutamate output varied with culture age. This requires that glutamate formation is controlled independently of the Krebs cycle. Although an increased ATP:ADP ratio, known to inhibit glutamate dehydrogenase in mitochondria, could be invoked to explain the results obtained in transition cells, this is not likely for AMP-treated log cells, as discussed earlier.

One could assume that the ATP:ADP ratio in the inner mitochondrial compartment in AMP-treated log cells was increased but that the increase was masked by opposite changes in other pools of adenine nucleotides, but such speculation seems unwarranted in the absence of measurements on variations in individual nucleotide pools.

| Table V |

| Effect of 6'-AMP on ATP and ADP levels in Tetrahymena. Cultures of 21 ml were grown in 500-ml Erlenmeyer flasks with shaking at 26°C for 17 hours. AMP, when present, was 3.6 mm. |

|                | N,F,Ni | ATP | ADP | ATP/ADP | ATP + ADP |
|----------------|--------|-----|-----|---------|----------|
|                |        | nmol/10⁶ cells | nmol/10⁵ cells |         |          |
| Log phase cultures |       |       |       |         |          |
| Control        | 6.3    | 3.7 ± 0.2 | 8.5 ± 0.4 | 0.43    | 12.2     |
| AMP-treated    | 5.7    | 3.3 ± 0.5 | 9.7 ± 0.8 | 0.35    | 13.0     |
| Transition phase cultures |       |       |       |         |          |
| Control        | 3.6    | 3.5 ± 0.2 | 7.6 ± 0.2 | 0.45    | 11.1     |
| AMP-treated    | 3.0    | 3.1 ± 0.1 | 7.5 ± 0.1 | 0.39    | 10.9     |

DISCUSSION

One test of any model is that it be able to account for data obtained when the system is perturbed. The model of metabolic flow used here (Fig. 3) was originally derived (3) for cells growing in the absence of added AMP. The ability of the model to fit the data obtained for cells treated with AMP or with tolbutamide (4), where there are changes in the rates of glyconeogenesis, lipogenesis, and oxidative metabolism suggest that the model is an adequate representation of the structure of intermediate metabolism in Tetrahymena, at least for the substrates and end products so far considered.

The present studies confirm the finding (1) that exposure of cultures to AMP for about 17 hours strongly inhibits glyoxylate oxidation and glyconeogenesis via the glyoxylate cycle. According to the metabolic scheme of Connett and Blum (11) peroxisomal isocitrate lyase splits isocitrate derived from the mitochondrial Krebs cycle into succinate, which returns to the mitochondria, and glyoxylate. Peroxisomal acetyl-CoA, derived primarily from acetate and secondarily from octanoate via peroxisomal β oxidation (12), condenses with glyoxylate via the action of malate synthetase. The peroxisomal malate so formed is either oxidized to oxaloacetate which can then enter the glyoxenogenic pathway via the cytosol phosphoenolpyruvate carboxykinase (11) or may re-enter the mitochondria where, presumably, it is oxidized in the Krebs cycle. The observation that 17 hours exposure to AMP strongly reduces glyoxylate oxidation and the incorporation of label from acetate into glyoxen from the peroxisomal compartment is consistent with the suggestion (1) that AMP treatment causes inhibition of malate synthetase or the transfer of malate from the peroxisomal compartment. In view of the change in RNA content and in the rate of synthesis of RNA in cells treated with AMP, it is possible that the reduction of carbon flux through the malate synthetase step may reflect a decrease in the amount of enzyme present or an inhibition of the activity of this enzyme. Although the analysis of intracellular flux rates as used in the present work cannot resolve this question, it does suggest that studies on factors controlling malate synthetase may be rewarding.

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It is noteworthy that although oxidative metabolism via the Krebs cycle decreased in both log and transition cells, the effect of AMP treatment on glutamate output varied with culture age. This requires that glutamate formation is controlled independently of the Krebs cycle. Although an increased ATP:ADP ratio, known to inhibit glutamate dehydrogenase in mitochondria, could be invoked to explain the results obtained in transition cells, this is not likely for AMP-treated log cells, as discussed earlier.

One could assume that the ATP:ADP ratio in the inner mitochondrial compartment in AMP-treated log cells was increased but that the increase was masked by opposite changes in other pools of adenine nucleotides, but such speculation seems unwarranted in the absence of measurements on variations in individual nucleotide pools.
cussed above. A similar comment applies to the decrease in rate of pyruvate utilization, \( V_r \). Taken together, these results imply that in addition to any regulatory effects of changing ATP:ADP ratios, AMP treatment must also cause some other control systems to change, and in a manner dependent on culture age, in *Tetrahymena*.

When cells growing without shaking were incubated with AMP for 17 hours, an increase in glycogen content occurred (1). In the present experiments, conducted on shaken and hence well aerated cultures, AMP treatment also led to an increase in glycogen content (data not shown), but caused a marked inhibition of glyconeogenesis from labeled acetyl-CoA. Since the data in Fig. 1 indicate that glyconeogenesis was already strongly inhibited by 6 hours after initiation of growth in the presence of AMP, it appears that there must have been an increase in glyconeogenesis from unlabeled precursors (not accounted for in this abbreviated model) and possibly a decrease in glycogenolysis early in the growth period. The marked change in RNA synthesis rate early in the growth period (Fig. 2) suggests that addition of AMP may cause a sequence of temporal changes in enzyme complement in *Tetrahymena*. Voichick et al. (15) have recently shown that a decrease in cyclic 3':5'-AMP in *Tetrahymena* correlates with an increase in glycogen content and of phosphoenolpyruvate carboxylase activity. In fat cells, adenosine was found to inhibit the accumulation of cyclic 3':5'-AMP that normally occurs in response to catecholamines plus theophylline (14). Since adenine, adenosine, and AMP all increased glycogen content in *Tetrahymena* (1), it is possible that they did so by inhibition of adenylate cyclase, as postulated for fat cells by Fain et al. (14).

It should be noted that Atkinson (16) has suggested that the ratio (ATP + 0.5 ADP) to (ATP + ADP + AMP) is the effective control parameter for several oxidative processes. Our conclusion that the ratio of ATP:ADP is not a crucial control parameter in *Tetrahymena* is not in conflict with Atkinson's view. Measurements of AMP content would be necessary to gain further insight into the role of adenine nucleotide ratios in the control of metabolism in *Tetrahymena*.

The present experiments have shown that the three acetyl-CoA pool model deduced earlier (3) can give a quantitative picture of the changes in intracellular flux patterns in response to AMP treatment, and thus emphasize the crucial role that compartmentalization plays in determining the metabolic response of a cell to alterations in its environment. The results indicate that the effect of AMP, in particular, is a complex time- and age-dependent shift in several pathways. A useful feature of this kind of analysis is that analysis of the changes in flux pattern suggest the types of experiments that should be performed in order to better understand the effects of AMP on metabolism.

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