Purine Metabolism During Development in the Cellular Slime Mold, *Dictyostelium discoideum*

by

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Submitted in Partial Fulmillment of the Requirements of the University Undergraduate Fellows Program

1978-1979

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April 1979

ABSTRACT

The studies reported in this paper examine the metabolic reorientation of purine nucleotide metabolism during the development of the cellular slime mold, Dictyostelium discoideum. There appears to be an energy oriented redirection of purine nucleotide pools during development and these changes have been evaluated. The slime mold maintains its ability to synthesize nucleotides de novo and raises intracellular pools. Metabolic perturbations may be induced by a variety of purine and pyrimidine analogues and anti-metabolites. All of these studies indicate that metabolic rearrangement of purine metabolism plays an integral role in the development of D. discoideum.

Introduction

The control of cellular differentiation is both an important and intriguing problem of biochemical genetics. The molecular mechanisms by which this complex process is initiated and regulated in eukaryotes requires a simple model system for developmental studies, as mammalian and amphibian systems present too many variables for precise biochemical analysis. An ideal model system for developmental studies is the cellular slime mould, Dictyostelium discoideum. This organism forms only three types of cells during its unique life cycle which can be easily induced and controlled in the laboratory: a free living amoeba differentiates into the stalk and spore cells. The life cycle is shown in Figure 1.

The free living amoeba grow on bacteria, usually Escherichia coli or Klebsiella aerogenes, in liquid or solid media sufficient for bacterial growth. They may also grow axenically (without other life forms) in complex media. The amoeba are attracted to the bacteria by a chemotactic response to folic acid, which is a metabolic by product of the bacteria (1). The amoeba will continue to grow by mitotic cell division as long as nutrients are present but upon nutrient deprivation they lose their chemotactic attraction to folic acid and begin to secrete cyclic SMP (2). The cells then secrete a heat-stable protein inhibitor of extracellular cAMP phosphodieterase (3) which allows cAMP levels to rise outside the cell. Subsequently chemotactic response to cAMP develops and the cells migrate towards a common aggregation center which is established by pulsating changes in cAMP concentrations. The cells come together in multicellular aggregates of about 10^5 cells $(4)_3$ in which each cell maintains its individual interfrity. The grex or

pseudoplasmodium, is formed by the secretion of a cellulose sheath which surrounds the aggregate. This cellulose sheath acts to exclude other amoeba and establishes organismal structure. The pseudoplasmodium may or may not begin a period of migration until suitable conditions for differentiation are found. During this time, the pseudoplasmodium respond to changes in humidity, temperature, and light (5). Subsequent development of cells is based upon the cell's position within the pseudoplasmodium (6). The first cells to arrive at the aggregation center appear to assume an interior position in the pseudoplasmodium and later become stalk cells (7). Cells that arrive later become prespore cells. Once cohesion is completed no significant shifting of position within the grex can be observed (8,9). After three to four hours the tip of the grex, containing pre-stalk cells extend downward through the rest of cells to contact the solid substrate (10). There cells form vacuoles and secrete a cellulose sheath. The main cell mass ascends the stalk, and prespore cells within the mass begin to encapsulate to form spores (11). The encapsulated spores can survive long periods of starvation, dehydration, and elevated temperature (12). The spores will germinate when favorable conditions exist, including a source of amino acids, as de novo protein synthesis is required (13). Upon germination the spore capsule splits longitudinally and a small, normal amoeba emerges to begin the life cycle over again.

Many biochemical changes underlife these morphological changes. Cellular metabolism is reoriented towards differentiation. The rate of oxygen consumption decreases (14), energy is obtained from amino acid oxidation, a resulting in a 50% loss of protein per cell (15). During

this time, several stage specific enzymes are produced and degradation and synthesis of RNA occurs (16).

Nucleotide metabolism may play an important role in the reorientation towards differentiation. Changes in the levels of nucleotides within the cell have been known to be one of the earliest metabolic changes during the early stages of differentiation, prior to other changes brought about by degradation of cellular protein and RNA. The changes in nucleotide metabolism are one of the first responses observed as a result of nutrient deprivation, and may provide a signal for the cell to begin differentiation.

The cell can obtain pyrimidine (uridine and cytosine) and purine (adenine and guanine) nucleotides from three sources for the synthesis of RNA and DNA. They may be recovered from the degradation of RNA, from sources outside the cell or they may be synthesized de novo. These studies evaluate purine metabolism during development of D. discoideum.

The major salvage pathway for purines converts free bases (adenine, guanine, or hypoxanthine) into the corresponding 5'-phosphates by specific purine nucleoside phosphoriboysl transferase (17, 18). A secondary pathway recovers purines as ribosides by specific nucleoside phosphorylases with later phosphorylation to nucleoside triphosphates (19). The majority of free purines are recovered by there salvage pathways (17), and thus they are essential for maintaining purine nucleotide levels within the cell (see Fig. 2). 5'-phosphoribosyl-1-pyrophosphate is a very important intermediate in the salvage of purine and pyrimidine nucleosides and in their de novo synthesis. The intracellular levels of PRPP may be an important factor in the regulation of de novo synthesis of purines (20), in fact, its availability may be a rate-limiting factor in this pathway (18).

The formation of PRPP is the first step in the de novo synthesis of purines. It is formed by the transfer of pyrophosphate groups from ATP to α-D-ribose-5-phosphate. This reaction is catalyzed by the enzyme phosphoribosylpyrophosphate synthetase (E.C. 2.7.6.1), which is inhibited by both ADP and GDP and is an important control point for de novo synthesis (21). In the next step, PRPP reacts with glutamine to that the amino group from glutamine displaces the pyrophosphate to yield glutamate and 5-phospho-B-D-ribosylamine. This step is catalyzed by the second important enzyme of de novo synthesis, PRPP amidotransferase (E.C. 2.4.2.14). Synthesis then proceeds through several steps to yield inosinic acid (IMP). This phosphorylated compound is the precursor for the formation of both adenylic acid (AMP) and guanylic acid (GMP). These two compounds are then further phosphorylated to yield ATP and GTP, which may then be incorporated into RNA, used as energy carriers within the cell, or converted to deoxyribosides for DNA synthesis.

Since PRPP is an important metabolite for the formation of both purines and pyrimidines, the enzyme catalyzing its formation has been extensively studied. Atkinson has suggested that PRPP synthetase is subject to partial control by cellular energy charge in E. coli (22). The allosteric control of this enzyme is quite complex and a variety of inhibitors have been described, including the uridine nucleotides (23).

The first enzyme unique to de novo synthesis of purines is PRPP amidotransferase, and it is also subject to regulatory controls in bacterial and mammalian systems. Various ribonucleotides have been shown to inhibit this enzyme from a variety of different sources (20). In E. coli

this enzyme is inhibited by ATP, ADP, AMP or by GTP, GDP, or GMP. Each of these two types of nucleotides apparently bind at separate allosteric sites on the enzyme (24). This inhibition is cumulative so that when both types of purine nucleotides are present at a high level, the enzyme is inhibited to a greater degree than by either type alone (24, 25). This enzyme, then, is the rate-limiting step of de novo synthesis of purine biosynthesis, and is the one studied in this research.

Synthesis of nucleotides produces nucleotide pools within the cells, and concentration of intracellular nucleotides can serve as a measure of the cell's metabolism. Shifts in the levels of specific nucleotides can thus reflect shifts in metabolism (26). Nucleotide levels are commonly represented in a form known as energy charge (27) which indicates the extent to which the ATP-ADP-AMP system is filled with high energy phosphate groups. Energy charge is calculated from the equation.

$$\text{Energy charge} = \tfrac{1}{2}(\text{ADP} + 2\,\frac{\text{ATP}}{\text{AMP}} + \text{ADP} + \text{ATP})$$

Atkinson has suggested that energy charge may be a major contorl factor in pathways that produce high energy phosphate groups. It certainly serves as an indicator of metabolic activity.

Charges in nucleotide metabolism may provide a signal for cellular differentiation, Dictyostelim discoideum was used in these studies because it represents a simple, but not necessairly primitive eukaryotic system. The levels of intracellular nucleotide pools were monitored during differentiation using high pressure liquid chromatography. Purine metabolism was studied by monitoring the first enzyme unique to de novo synthesis of purine PRPP amidotransferase by an in vitro enzyme assay. The activity of

of this enzyme was monitored at several different stages of development to determine if any changes in de novo synthesis of purines took place. Purine and pyrimidine analogs were tested to see what, if any effect they would have on development.

Materials and Methods

Growth Procedures

Strain AX2 of Dictyoslelium discoideum was cultured axenically in a nutritive medium consisting of 14.3 g. of bacteriological peptone (Oxiod), 7.2 g of yeast extract (Oxoid), 1.28 g of Na_2HPO_4, 0.486 g of KH_2PO_4, and 15.4 g of dextrose per liter of distilled water. Amoeba introduced into this medium were cultured in Erlenmeyer flasks at 22°C. with shaking to ensure maximum aeration. The amoeba will multiply to a concentration of 5×10^6 cells/ml within 3 days (determined by heamocytometer count) at which time 0.1 ml is transferred into a flask containing fresh medium. Alternatively, D. discoideum could be grown on agar plates containing nutrient sufficient for bacterial growth. The amoeba grow on the bacteria until the bacteria supply is exhausted, at which time they will differentiate into fruiting bodies. Spores were then collected using a wire loop and placed in a solution containing .09% NaCl and 5% streptomycin. Spores could be frozen in this solution at -15° to -20°C indefinitely for later use. Spores were germinated by heat shocking at 45°C for 30 minutes, then placed in axenic media as above. They would multiply to 5×10^6 cell/ml within 7-9 days.

Induction of Differentiation

Differentiation was induced by placing actively growing amoeba (concentrations less than 5×10^6 cells/ml under non-nutritive conditions. Amoeba were collected from the axenic medium by centrifugation and then resuspended in pad buffer (1.5 g KCl, 0.5 g $MgCl_2$ per liter of distilled water) at a concentration of 10^8 cells/ml. The cells were then spread evenly across developmental plates. (These were disposable petri dishes

containing two layers of filter paper in the bottom with a Millipore filter (HAWP 00 25) on top of them. Another piece of filter paper was taped to the underside of the lid. These were saturated in pad buffer to maintain adequate levels of humidity. Between 5 - 20 x 10^7 cells (0.5 ml to 10 mls) were spread on the millipore filters, depending on the size of the plate. Smaller plates were used for analogue studies and nucleotide pool extractions. The larger plates were used for enzyme analysis. Excess liquid was removed from the bottom of the plates to ensure proper differentiation. The lids were taped shut to ensure high humidity within the plates; they were then placed in an incubator at 22°C with an overhead light. The entire developmental sequence (see Fig. 1) takes place in 24 hours under these conditions.

Analog Studies

The effects of various purine and pyrimidine analogues, metabolic intermediates, and drugs on cellular differentiation were tested using the developmental plates described above. The pads were saturated in a solution containing the analogues at a concentration of 5.0 mM. The purine compounds tested were: guanine, adenine, isosine, xanthine, hypoxanthine, guanosine, adenosine, xanthosine, 2-mercaptopurine, azaserine, 6-azaguanine, 5-flurocytosine, 4,5-diamino pyrimidine, 5-diazouracil, 5-diazouidine, 5-aminoaracil, 5-flurouracil, 5-bromouracil, 5-flurouridine, 5-bromouridine, isocytosine, aminopterin, methotrexate, orotate, dihydroorotate, and barbiturate. Morphological development was monitored over the twenty-four hour cycle with observations being made every 3 hours. (see Table 1) Samples containing analogues were compared to control plates at these times. Several classes of developmental response were observed: no effect, absence of aggregation, and accelerated development.

Enzyme Extract Preparation

Preparation of crude protein extract for enzyme analysis from bacteria was modified from the procedure of Nierlich and Magaianik (28). Salmonella typhimurium strain LT2, was grown in minimal Davis media, harvested by centrifugation (5000 RPM for 10 minutes). The cells were resuspended in one tenth volume of phosphate buffer (0.3 M, pH 7.5) and recentrifuged at 5000 RPM for 10 minutes to wash them. The cells were resuspended in the same buffer. All cells are disrupted by sonification using a Branson Sonifier Cell Disrupter Model W185 (Head Systems, Ultrasonics Inc., Plainview N.Y.). For bacterial cells, 4 thirty second blasts at 75-85 watts of power were sufficient. The cell mixture was kept in an ice bath at all times to minimize enzyme denaturation. After sonication, the cell mixture was centrifuged for 30 minutes at 18000 RPM to remove cellular debris. The supernatent was then desalted by passing it through a Sephadex G-25 column to remove ammonium ions, which inhibited the enzyme. Extracts from Dictyostelium discoideum were made in the same manner, however only 2 twenty second blasts of sonication were required to disrupt the more fragile cells. Extracts could be used immediately or frozen at -20°C, where they would retain activity for up to one week.

Assay Procedures

Phosphoribosylpyrophosphate amidotransferase (EC2.4.2.1.4) was assayed according to the procedure of Nierlich and Magasanik (28). PRPP amidotransferase was assayed by measuring the appearance of the 3-acetyl analog of NAOH. This is a two step reaction in which the amino group from glutamine is transferred to PRPP via the action of PRPP amidotranferase (step 1) and the glutamate thus formed is then oxidized to α-ketoglutarate by the action of glutamate dehydrogenase (step 2) with the concomitant reduction

of NAD:

The 3-acetyl analogue of NAD was used in order to push the equilibrium of the reversible second step of this reaction to the formation of α-ketoglutarate. This reaction was monitored in 1 ml quartz curvettes at 30°C (10 mm path length), containing: 0.1 ml tris buffer (6 μmoles/ml), 0/05 ml $MgCl_2$ (12 μmole/ml), 0.15 ml H_2O, 0.1 ml 3-acetyl NAD (12 μmoles/ml), 0.1 ml PRPP (17 μmole/ml), 0.2 mls crude enzyme extract and 0.2 mls of glutamte dehydrogenase. This was allowed to equilibrate at temperature for 10 minutes, then 0.1 ml of glutamine (100 umoles/ml) was added to start the reaction. The appearance of 3-acetyl NAOH was monitored at 363 manometers using a Gilford model 250 kinetic spectrophotometer. These purification and assay procedures was the result of much trial and error using extracts from bacteria (Salmonella typhimuium type LT2 and E. coli type B/r) before stage specific assays using Dictyostelium discoideum could be attempted.

Stage Specific Assay

PRPP amidotransferase was assayed at various stages of development of D. discoideum as described above. A certain number of cells were placed on large developmental plates and extracts made at 0, 4, 8, 12, and 27 hour stages. Protein estimations of these extracts were made using the Bio-Rad protein assay (Bio-Rad Laboratories). To 0.1 ml of the protein solution was added 5 mls of Bio-Rad dye (diluted 1 part to 4 parts distilled H_2O. Absorbance was measured 595 monometers after blanking against 0.1 ml H_2O in 5 mls dye. PRPP amidotransferase activity is reported as nmoles 3-ac NADH/minute/mg protein/ml.

Analysis of Nucleotide Levels by HPLC

Cells were collected form various stages of development by washing them off of differentiation plates, centrifuging and resuspending them in pad buffer. The cells are then centrifuged at 5000 PRM for 2 minutes, resuspended in pad buffer and counted by hemocytometer. The number of cells was adjusted to 5×10^7 cells and then recentrifuged. The cell pellet was then resuspended in 1 ml trichloroacetic acid (TCA). This disrupts the cell and precipitates macromolecules. After allowing the TCA extract to sit for 30 minutes, the samples are centrifuged at 15000 RPM for 15 minutes to remove all cellular debris and precipitated macromolecules. The resulting supernatent contains free nucleotides. The samples were passed through a millipore filter and neutralized with an equal volume of amine-freon solution (0.7 M amine in freon). This solution was mixed thoroughly and the amine-freon solution allowed to settle out. The aqueous layer, containing the nucleotides, was collected and frozen at -20°C until HPLC analysis could be attempted (39).

A Waters Associates ALC GLC 202 high pressure liquid chromatograph equipped with a model NGK septumless injector, two model 6000 solvent delivery pumps, a model 660 solvent programmer, and a Partsil-10 Sax column was used to separte the nucleotides. Resolution of the nucleotides was achieved using a linear solvent gradient ammonium dihydrogen phosphate at a flow rate of 2 ml/minute. A model 440 absorbance detector was used to detect the eluting nucleotides. Absorbance was measured at 254 mm and recorded on a recording chart. Fifteen µl of a standard solution including adenine, guanine, cytidine and uridine monophosphates, diphosphates, and

triphosphates was injected to determine standard retention times. Experimental extracts were then injected to determine the levels of nucleotides at each stage. All HPLC work was done by Mr. Leonard Schronk of the Agriculture Analytical Laboratory, Texas A&M University

RESULTS

Analogue Studies

Several purine and pyrimdine analogues disrupted normal development of D. discoideum as can be seen in Table 1. Some promoted and accelerated development and others inhibited or slowed the developmental sequence. The introduction of pyrimidines which enter the biosynthetic pathway accelerate the developmental process. In addition, aminopterin and methotrexate (folate analogues, 29) disrupted development. Orotate and dihydroorotate are intermediates in de novo biosynthesis and accelerated development. Barbiturate is a product of oxidative degradation of uracil and can enter into the synthesis of new pyrimidines. Barbiturate also accelerates development. The fourth compound which accelerated development was allopurinol, a drug known to interfere with purine metabolism (30) as an analogue for hypoxanthine, an intermediate in purine biosynthesis. Thus, a variety of purine and pyrimidine metabolites and antimetabolites alter development in the cellular slime mold.

Stage-specific Assay of phosphoribosylpyrophosphate amidotransferase (PRPP amido transferase, EC. 2.4.2.14).

The activity of PRPP amido transferase during 5 different morphological stages of development were determined as described in materials and methods. The activity of the enzyme is expressed as nmoles of 3-acetyl NADH produced per minute per mg protein. The increase in absorbance at 363 nmeters indicates the reduction of 3-acetyl-NAD to 3-acetyl-NADH, and thus is an indirect measurement of the activity of the enzyme. The enzymatic activity is maintained throughout development and increases dramatically at the twelve hour stage of development. Activity returns to previous levels by the end of development. These results suggest that de novo synthesis of purines is maintained during development. Previous work has shown that aspartate transcarbamylase (ATCase, EC.2.1.3.2) activity disappears after

aggregation (Fig 4.). These results have been repeated recently and verified. This enzyme is a unique regulatory enzyme for the de novo synthesis of pyrimidines, and the loss of activity suggests that the pathway is not continually used during development.

Levels of Nucleotides During Development.

The levels of intracellular nucleotides were analyzed using high pressure liquid chromatography (HPLC). The nucleotides were separated by a solvent gradient on a Partacil-10 SAX column and quantitated by spectrophotometric absorbance at 254 nmeters. Retention peaks indicate each nucleotide relative to standard retentions and their heights are proportional to the concentrations of the pool. The nucleotides elute from the column in three distinct groups: first the mono-phosphates, then the diphosphates, and finally the triphosphates. Samples were taken every three hours during the developmental sequence and analyzed by HPLC. Chromatographs from the amoeba prior to starvation (Fig. 5) and from the twelve hour aggregation stage (Fig. 6) show that the fluctuations in nucleotide pools are quite complex:

1. absolute levels of ATP and GTP increase,
2. the levels of triphosphates increase relative to the mono and diphsophates,
3. the levels of purines increase relative to the pyrimidines
4. some nucleotides appear in the 12 hour sample which were not present in the amoeba (eg. inosine-monophosphate (IMP) and a UDP-sugar).

The UDP-sugar is probably used for formation of the cellulose sheath, while IMP is a central intermediate in purine biosynthesis. The presence of UDP-sugar is clearly associated with the formation of stalk cells and the sudden appearance of IMP indicates that purine metabolism is altered at this stage of development. A graph of the increased intracellular levels of IMP and UDP-sugar over the various developmental stages (Fig. 7) show that the levels of these two molecules increase at very specific times. The distinct fluctuations of ATP and GTP can

be more clearly seen when their pool concentrations are presented relative to the total developmental cycle (Fig. 8). ATP shows sharp increases at the four and twelve hour stages. This fluctuation does not occur when the amoeba are are prevented from forming tight cell-cell contact by shaking is buffer-salts. When starved under non-nutriative conditions without cell-cell contact, ATP and GTP pool levels fall precipitously (Fig. 9). This shows that ATP and GTP levels are precisely controlled when development is allowed and not maintained otherwise.

DISCUSSION

Changes in nucleotide metabolism during the development of Dictyostelium discoideum were the subjects of the research presented in this paper. The research was organized into three basic parts. The effects of nucleotide analogues were examined by exposing the myxamoeba to moderate levels (5mM) of base analogues, metabolic intermediates, and drugs during development. The developmental cycle was monitored in the presence of these compounds for morphological disturbances. Secondly, de novo synthesis of purines was examined by measuring the first enzyme unique to that biosynthetic pathway during the developmental sequence. Finally, intracellular nucleotide levels were measured directly by high pressure liquid chromatography (HPLC). The results from these studies suggest that nucleotide synthesis is indeed effected during development and may be a possible regulatory mechanism for cellular differentiation.

The presence of a variety of nucleoside analogues disrupted morphological development. Those compounds which inhibited development may act by inhibiting or disrupting specific enzymes in nucleotide synthesis and thus disrupting metabolism. The analogues of uracil and uridine (5-aminouracil, 5-fluorouracil, and 5-fluorouridine, for example) may inhibit aspartate transcarbamylase (EC 3.1,3.2) which is an important enzyme in the de novo pathway of pyrimidines (31). Perhaps purine analogues (6-azaguanine, 6-azaadenine, 6-mercaptoguanine) could be inhibiting specific enzymes such as phosphoribosylpyrophosphate synthetase (EC 2.7.6.1) or phosphoribosylpyrophosphate amidotransferase (EC 2.4.2.14) which are early regulatory enzymes involved in the de novo synthesis of purines. These suppositions could be tested directly using an in vitro assay for enzymatic activity. Several of the compounds which accelerated development (orotate, dihydroorotate, and barbiturate) are intermediates in de novo pathway for pyrimidine biosynthesis or involved in oxidative degradation of pyrimidines. Since we know that the aspartate transcarbamylase, the enzymes regulating de novo synthesis, disappears

during development, these intermediates may accelerate development by allowing continued synthesis of pyrimdines during the early stages of development. The folate analogues (aminopterin and methotrexate) inhibited development. These compounds are known to inhibit the formation of DNA and slow the growth of cancer (32), and thus it might be suggested that the disruption of DNA synthesis might perturb later cellular development. In addition, however, these compounds inhibit tetrahydrofolate reductase and interfere with folate metabolism. Allopurinol, an analogue of hypoxanthine, accelerated development. This compound is known to inhibit xanthine oxidase, an enzyme involved in the salvage/reutilization of purines (33). This inhibition would tend to maintain elevated levels of purine nucleotides - perhaps essential for normal development. This part of the research is preliminary and the results must be considered cautiously. Nonetheless, these studies would seem to indicate that a variety of different control points may be involved in the overall coordination of pool levels of nucleotides during development.

The next stage of the research involved a study of a regulatory enzyme in the pathway for the de novo synthesis of purines, phosphoribosylpyrophosphate amidotransferase, the second enzyme in the biosynthetic scheme. Preliminary observations reveal that this enzymes remains active throughout the developmental cycle. In fact, it may even increase during the early stages and it is most active at the twelve hour stage when individual cells within the cell mass are undergoing drastic developmental changes into spore and stalk cells (34). Increased de novo synthesis of purines indicates that the cells have an increased demand for purines. The purine triphosphates (ATP and GTP) are important energy carriers within the cell, and the morphological changes of the cell require increased energy and thus elevated de novo synthesis may be required. The fact that de novo synthesis of purines does not disappear during development, in contrast to pyrimidine biosynthesis which does disappear, indicates that the purine requirement increases relative to the pyrimidine requirement.

In fact, purine nucleotides do increase dramatically during development as measured directly by high pressure chromatography. ATP and GTP levels show stage-specific increases associated with two major developmental points. ATP pools, and GTP pools to a lesser extent, rise sharply at four hours (the beginning of aggregation) and again at twelve hours (the formation of the differentiating cell mass capable of migration). These increased levels of ATP indicate that the cell may require an increased supply of energy during these times or perhaps a regulatory signal. After the twelve hour stage the nucleotide levels drop sharply. The spore and fruiting body do not maintain high ATP and GTP pools. If the energy charge within the cells are calculated during these changes, it can be shown that the energy available slowly climb throughout late aggregation and then suddenly decline during the slug and spore stages. This indicates that the cell has a specific need for increased available energy and tha that a reduced need in encountered after aggregation. Adkinson has suggested that adenylate energy charge may be one of the most important regulatory signals within a cell, but these studies do not indicate whether these changes are cause or effect.

Cell-cell contact on a solid substratum is essential for the metabolic reorientations described above. If amoeba are starved and resuspended in a shaking culture, nucleotide pools disappear rapidly and differentiation does not occur.

Purine nucleotides were observed in much higher concentrations than the pyrimidine nucleotides during all stages of development. This is somewhat expected since ATP and GTP are important energy components and regulatory molecules within the cell (37), however, the extent of differences between purines and pyrimidines is unexpectly high. The fact that the cell will maintain <u>de novo</u> synthesis of purines at great expense to the cell (38), even during starvation stages, indicates

that these molecules are essential for the metabolic processes which underlie cellular differentiation. Finally, the appearance of specific intermediates of nucleotide biosynthesis (eg. IMP, inosine monophosphate) at specific times during the developmental sequence indicates precise regulation of the process. The sudden increase in PRPP amidotransferase at the twelve hour stage, coupled with the apppearance of IMP and increased levels of ATP and GTP suggest that a major reorientation of purine metabolism occurs. It may be that this shift in metabolism acts as a signal for continuing cellular differentiation.

It is not remarkable that the enzymes needed for de novo synthesis of purines retain activity throughout development in spite of the loss of total cellular protein (35). The nature of metabolic flux through the pathway is continuing and the nature of allosteric regulatory controls over purine biosynthesis are being examined. Purine metabolism clearly occupies a central position in the reorientation of cellular metabolism during development of D. discoideum.

Acknowledgments

I would like to thank Dr. James Wild for his patient and diligent help. His guidance and enthusiasm for his work has been an inspiration to me. I would also like to thank Melinda Wales for her uncounted hours of work with the nucleotide pools.I wish them the best of luck in their future research endeavors.

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Figure 1. Life cycle of Dictyolstelium discoideum. Upon starvation individual cells aggregate to form a multicellular psuedoplasmodium. Individual cells differentiate into specialized spore and stalk cells to form the mature fruiting body.

Figure 2. Purine metabolism. Purine nucleotides available to cell by de novo synthesis, salvage of bases from degraded RNA and exogeneous sources. An enzyme unique to de novo synthesis, PRPP amidotransferase (PRPPATase) was monitored over the course of the life cycle

Table 1. Effect of nucleotides on development. Various pyrimidine and purine analogs were present extracellularly and their effects on morphological development were monitored over the life cycle.

Figure 3. PRPP amidotransferase activity was followed at five stages of development by monitoring production of 3-acetyl NADH. This enzyme, unique to de novo purine synthesis, retained activity throughout development.

Figure 4. Aspartate transcarbamylase activity was monitored during development in an earlier study. This enzyme regulates pyrimidine de novo synthesis and its activity disappears after the aggregation stage.

Figure 5. HPLC chromatograph of nucleotide levels at the amoeba stage. Diphosphate levels are higher than those for triphosphates and purine nucleotides are in higher concentrations than pyrimidine nucleotides.

Figure 6. HPLC chromatograph of nucleotide levels at the 12 hour stage of development. Dramatic increases in the levels of ATP and GTP over those of the amoeba stage can be seen. IMP and a UDP-sugar appear at this stage.

Figure 7. Graph showing sudden fluctuations in the levels of IMP and the UDP sugar over the course of development. IMP is a central intermediate in purine biosynthesis and may indicate increased activity of purine synthesis.

Figure 8. Graph of nucleotide triphosphate levels over the course of development. Increases in ATP and GTP are seen at the 3 hour and 12 hour stages. After 12 hours nucleotide levels drop sharply.

Figure 9. Graphy of nucleotide levels in cells prevented from undergoing differentiation ATP and GTP levels drop sharply when placed under stavation conditions without cell-cell contact. This clearly shows a relationship between nucleotide levels within the cell and cellular development.

figure 1

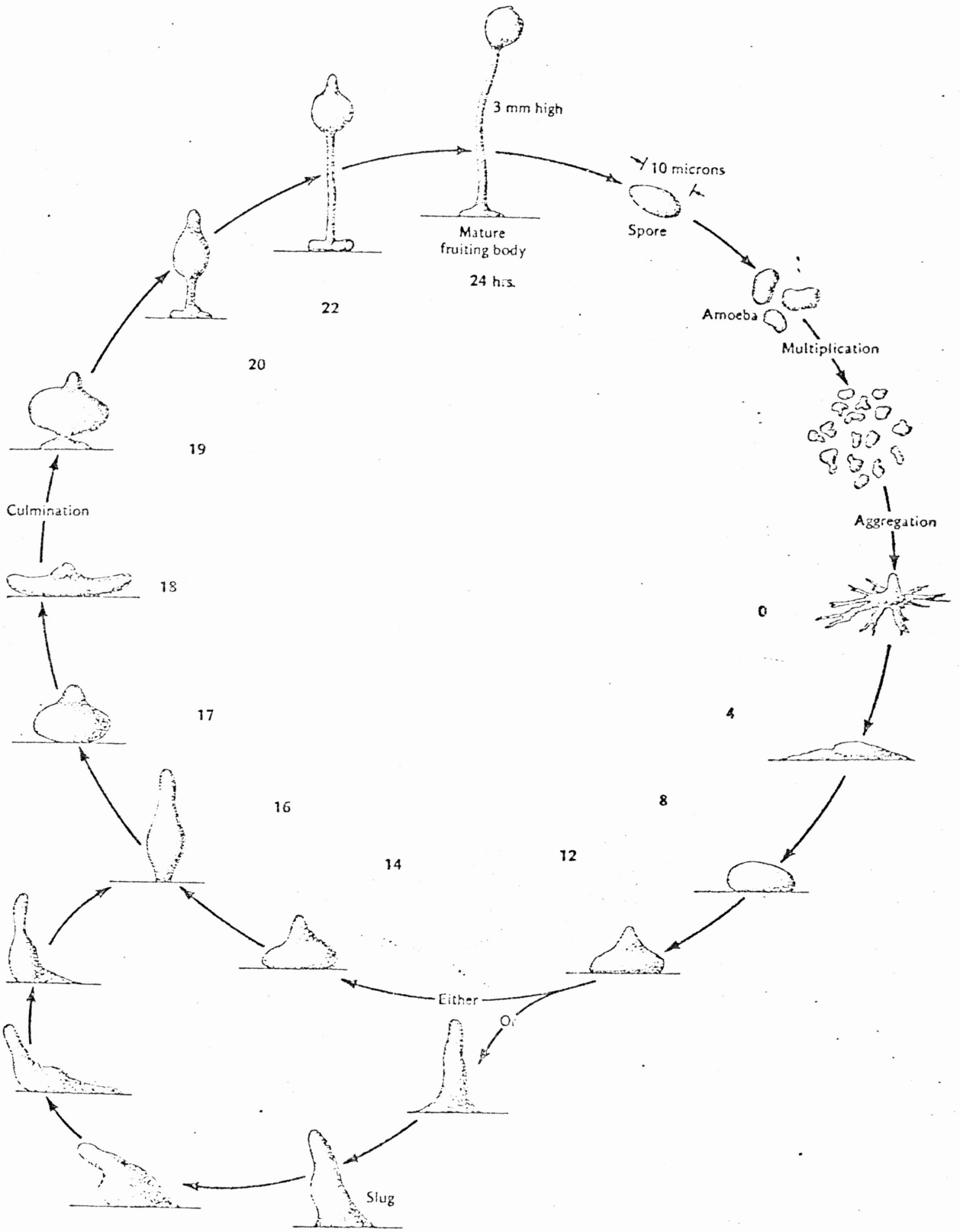

Figure 1 - Strickberger, M.W., 1976, Genetics, Macmillian & Company Inc. New York

Figure 2.

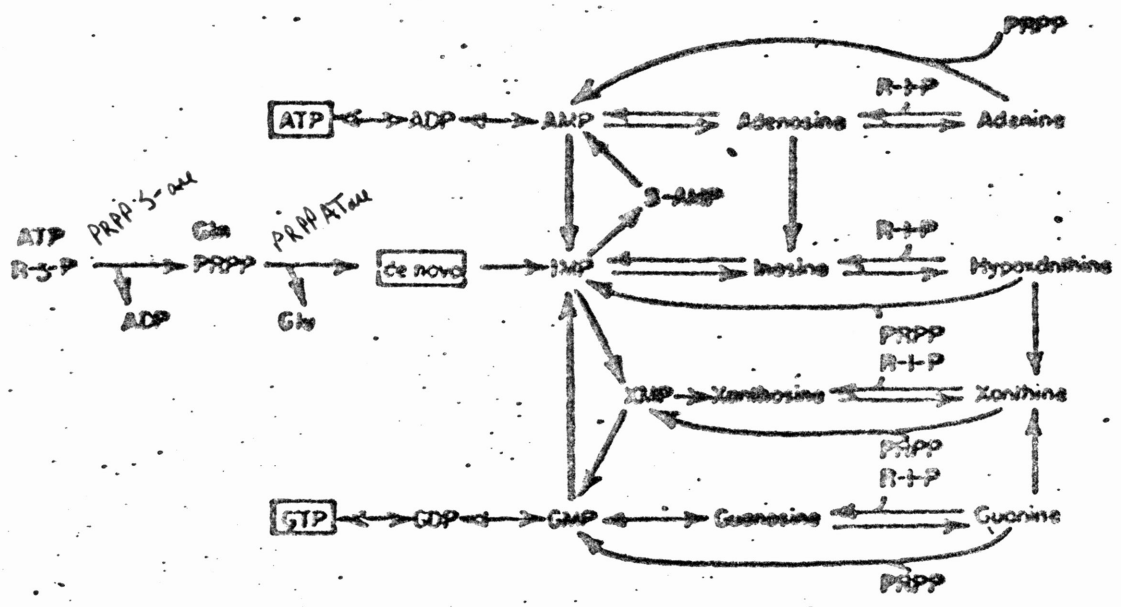

Fig. 4. GENERALIZED PURINE METABOLISM

Table 1.

EFFECT OF PYRIMIDINES ON MORPHOGENESIS - D. discoideum

No Alteration	No Aggregation	Accelerated
Thymine	5-aminouracil	Orotate
Cytosine	5-flurouracil	Dihydroorotate
Uracil	5-bromouracil	Barbiturate
Uridine	5-flurouridine	
Cytidine	5-bromouridine	
deoxyuridine	Isocytosine	
deoxycytosine	aminopterin	
deoxythymidine	methotrexate	
6-azauridine		
6-methyluridine		
6-azathymidine		
5-flurocytosine		
4,5-diamino pyrimidine		
5-diazouracil		
5-diazouridine		

EFFECT OF PURINES ON MORPHOGENESIS - D. discoideum

No Alteration	No Aggregation	Accelerated
Guanine	6-azaguanine	Allopurinol
Adenine	6-azaadenine	
Inosine	6-mercaptoguanine	
Xanthine		
Hypoxanthine		
Guanosine		
Adenosine		
Xanthosine		
2-mercaptopurine		
Azaserine		

Figure 3.

PRPPATase ACTIVITY

nmoles 3 acNADH/min./mg.protein/ml.

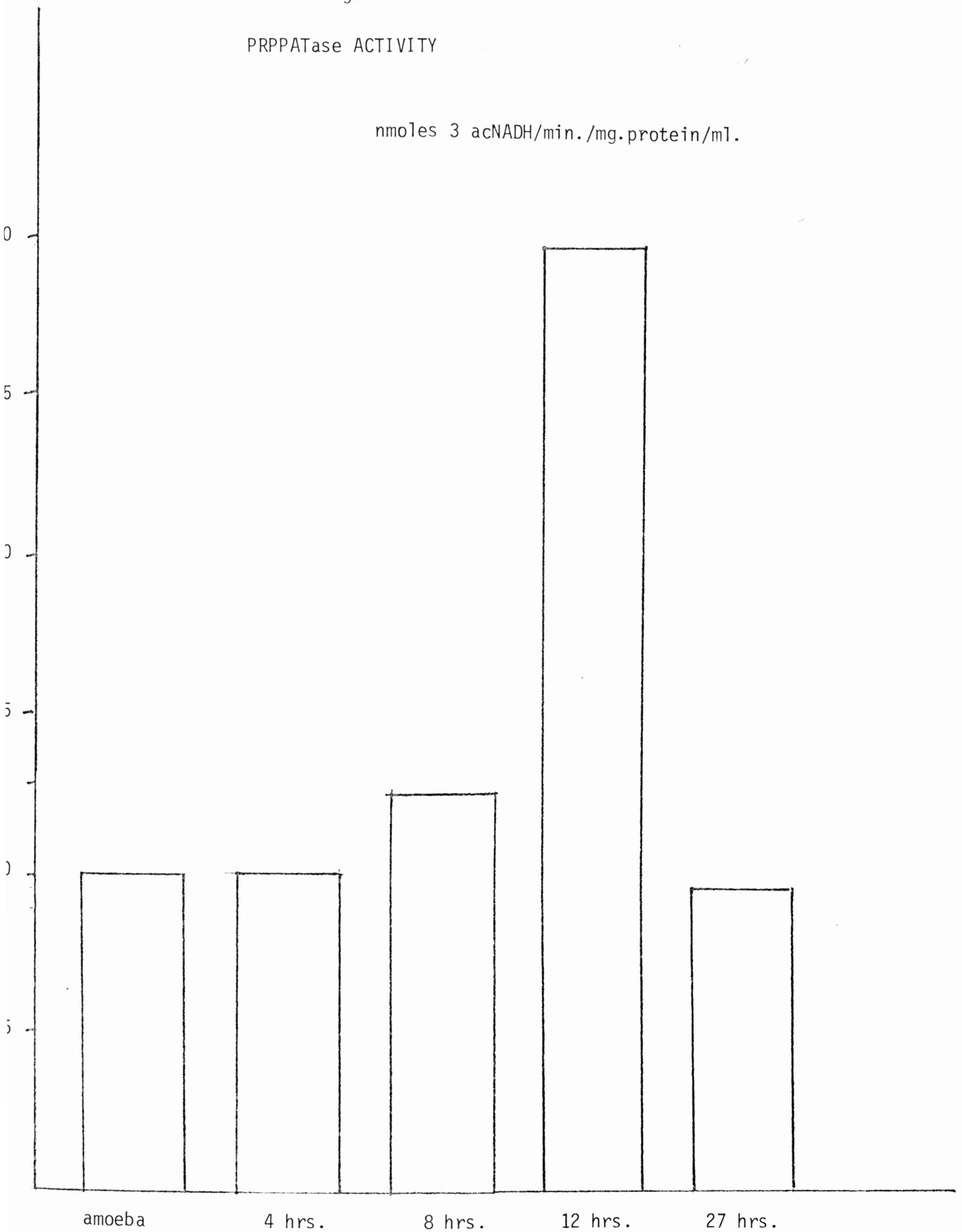

Figure 4.

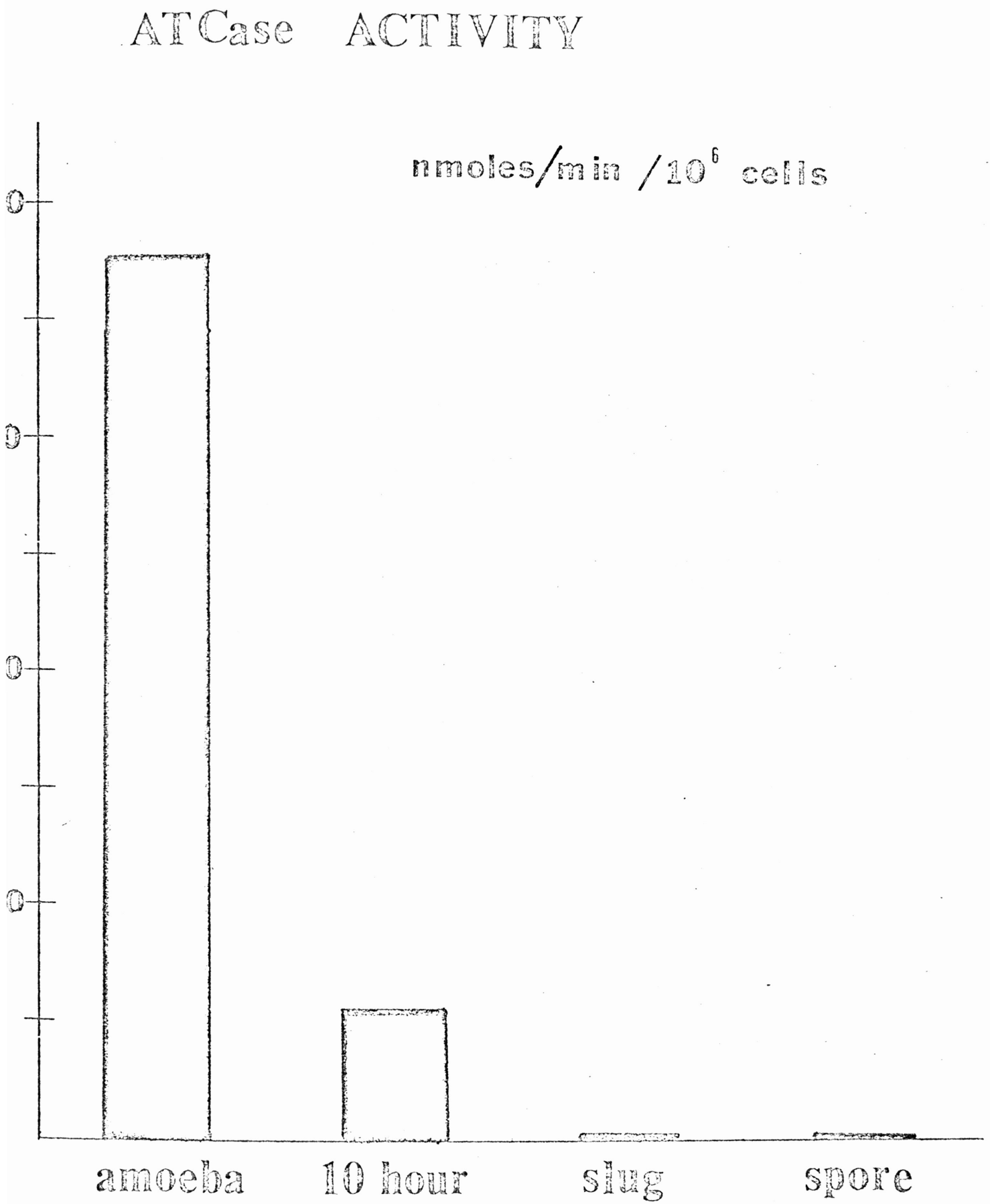
ATCase ACTIVITY
nmoles/min /10^6 cells
0
0
0
0
amoeba
10 hour
slug
spore

Figure 5.

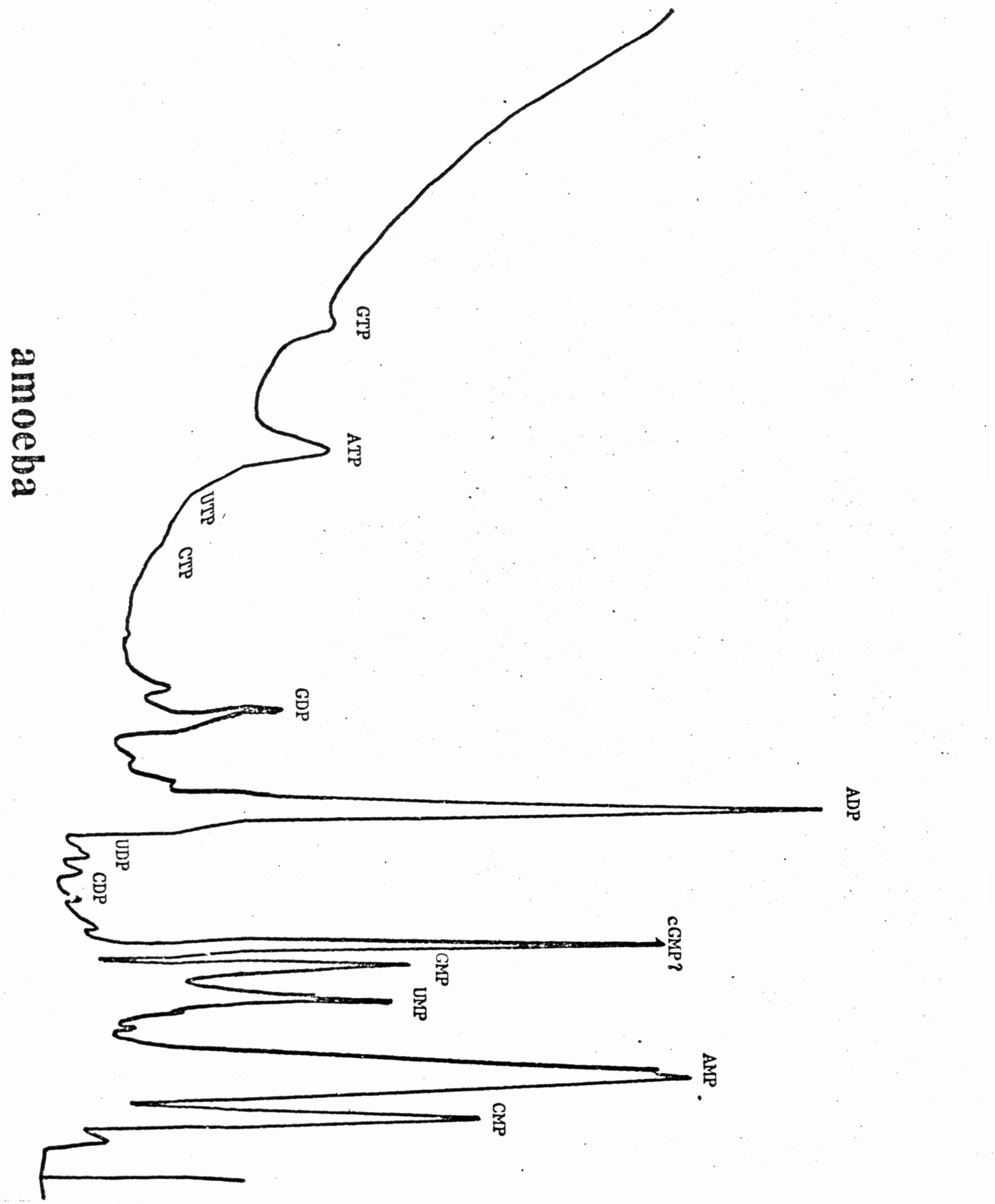

Figure 6.

1-23-79
developmental
12 hrs
100ul

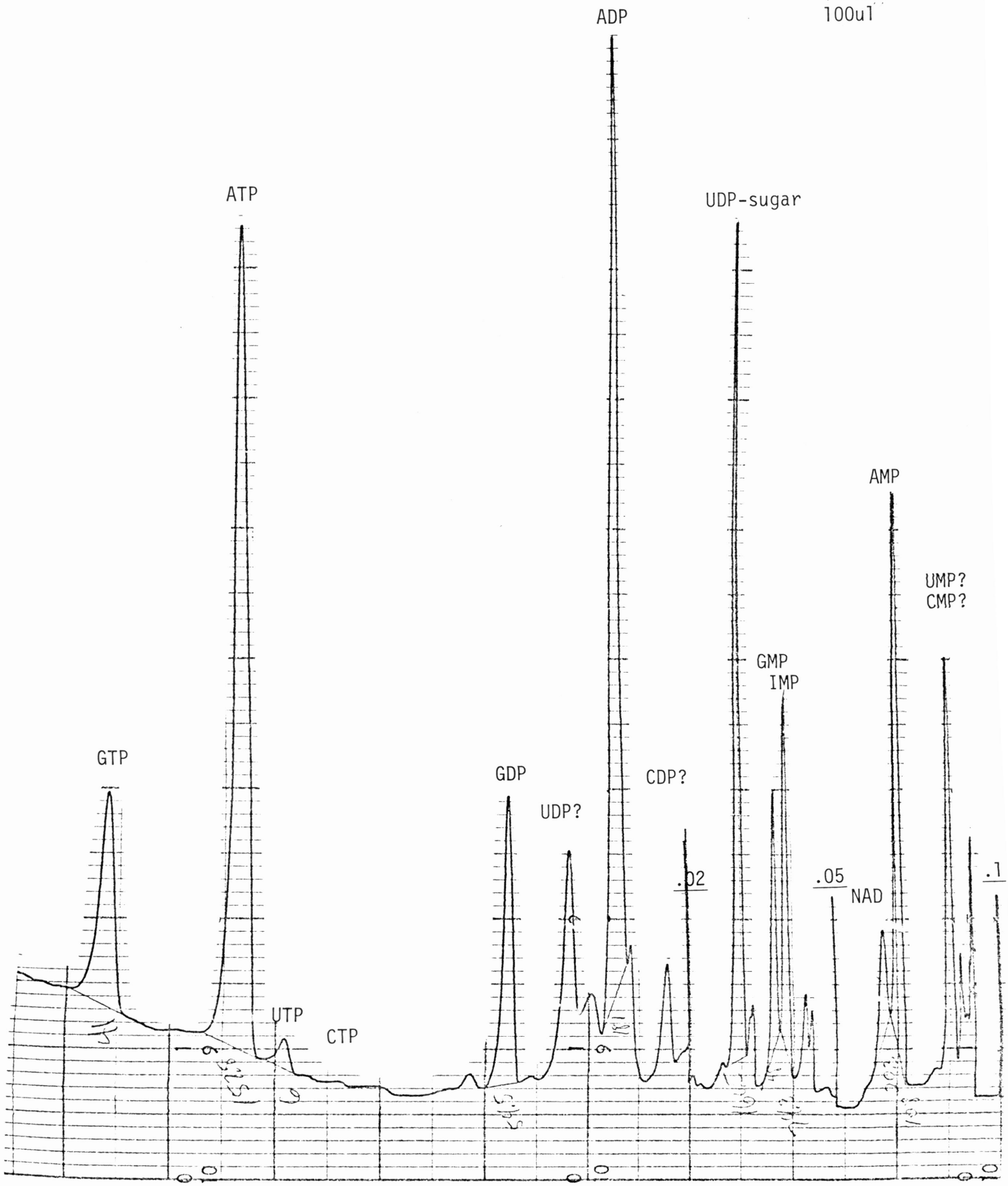

Figure 7.

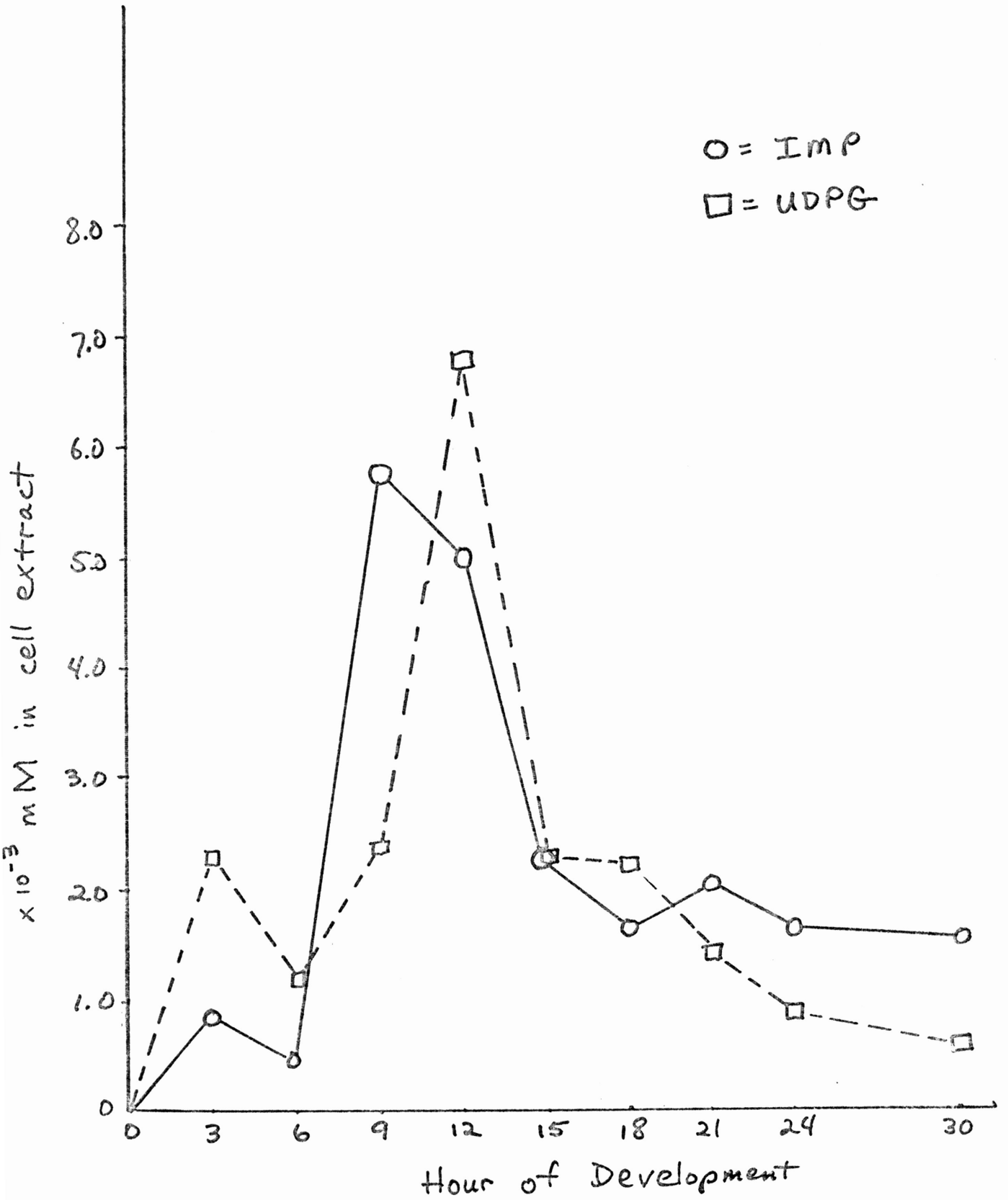
○ = IMP
□ = UDPG
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
$\times 10^{-3}$ mM in cell extract
0
3
6
9
12
15
18
21
24
30
Hour of Development

Figure 8.

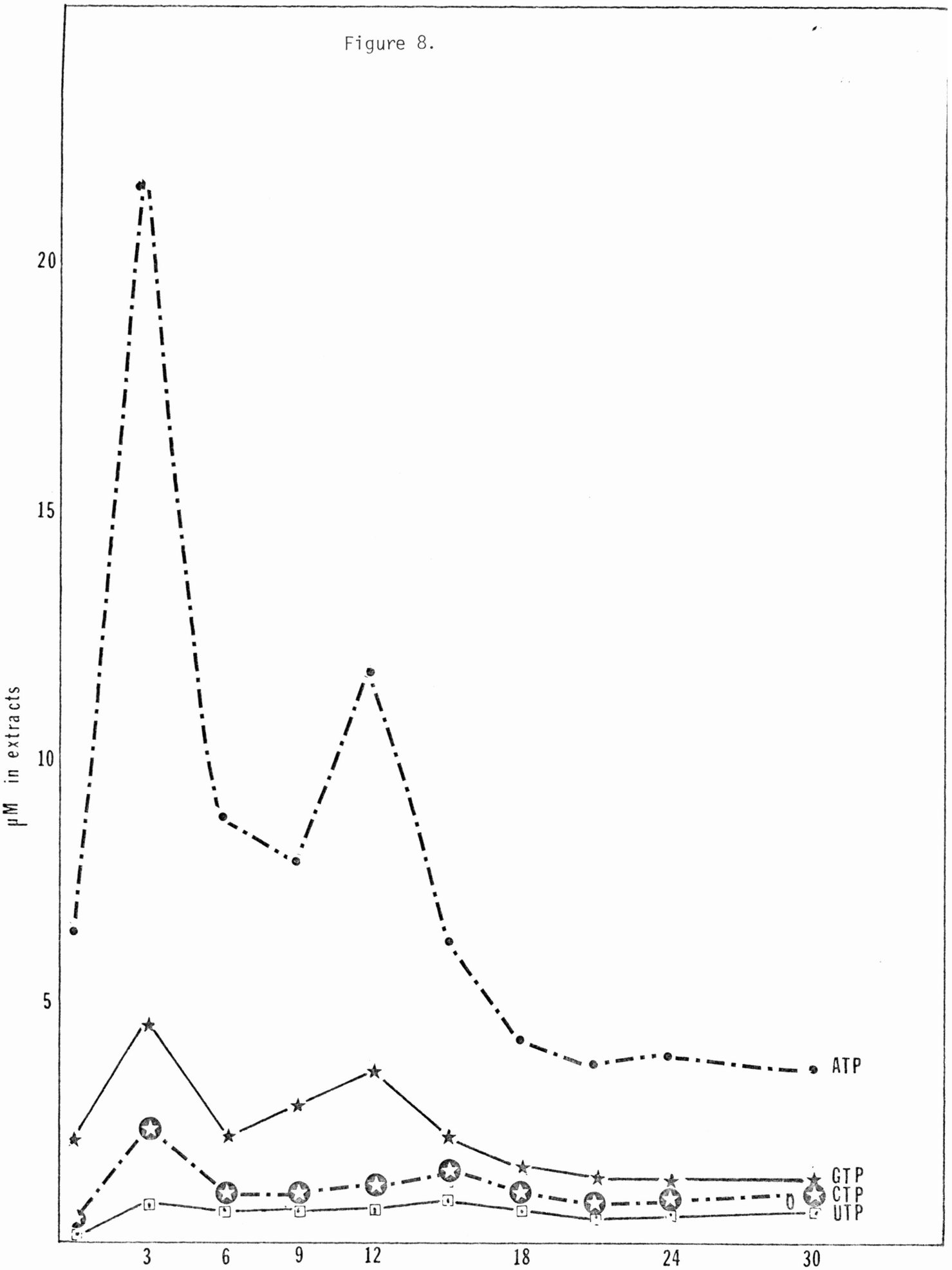

Figure 9.