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MACRONUTRIENT USE IN AXENIC CULTURES OF PARAMECIUM

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ABSTRACT: Axenic paramecium cultures are either protein-lipid or protein-carbohydrate based with respect to macronutrient components. In the first case, Fok, et al. (1981) determined that lipids are used rapidly and completely whereas 75% of the protein remains at the end of stationary phase. In the study reported here, analysis of the protein-carbohydrate utilization patterns showed that carbohydrate is used completely while much (70-75%) protein remains at the end of the growth cycle. Several experiments aimed at elucidation of the mechanism of the necessary role of the protein components are reported.

INTRODUCTION

Three species of *Paramecium* have received the most attention in nutritional studies: *P. tetraurelia, P. caudatum,* and *P. multimicronucleatum.* The corresponding media developed for these species have the following characteristics:

Species	Caloric Component-macronutrient	Name
1. *P. tetraurelia*	Proteose-peptone, lipid rich	Soldo's Crude Medium
2. *P. caudatum*	Protein fraction from peas	Lilly's Medium
3. *P. multimicronucleatum*		
	1. Non-dialyzable fraction from yeast-protein, polysaccharides	Johnson's NDF Medium
	2. Pure protein, pure glycogen	Johnson's Chemically Defined Medium

Most of the routine culture of axenic *Paramecium* has been with Soldo's crude medium of which all components may be purchased readily and are relatively inexpensive.

Fok, Allen, and Kaneshiro (Fok et al., 1981) reported the processing of macronutrients in Soldo's crude medium by *Paramecium multimicronucleatum.* They found that lipids are used rapidly and completely whereas the protein component is only 25% utilized at the end of stationary phase. In their experiments, stationary phase began at 6-8 days as depletion of the lipid was completed. Proteinaceous material is provided by the basal medium and complex lipids are provided by the sheep-brain cephalin fraction.

This study has analyzed some biochemical changes in Johnson's chemically defined medium (Johnson et al., 1980), namely macronutrient utilization by *Paramecium multimicronucleatum.* Only eighteen of the twenty protein amino acids are provided in the amino acid mixture; glutamine and cysteine are not present. The protein may be one of a number of purified proteins: albumins, globulins, globins, inactivated enzymes [catalase, urease, trypsin, chymotrypsin, pepsin], lectins and so forth. The glycogen

may be from various sources, but all of this work has been done with mussel (*Mytilus edulis* , Type VII, Sigma G-1508) glycogen, which is the best glycogen of those tried (Johnson et al., 1980; and unpublished observations).

MATERIALS AND METHODS

Cell culture

Cells were *P. multimicronucleatum,* purchased from Evergreen Biologicals in Seattle, WA, converted to axenic status with washing in rifampicin (50-100g/ml)-containing medium and have been grown axenically on defined medium since January, 1989. For these experiments, the cells were cultured on Johnson's defined medium in DeLong flasks. Other axenic lines of paramecia are those maintained in this laboratory for several months to years. Ovalbumin (5x recrystallized, 10mg/ml) was obtained from K&K Laboratories. Other chemicals were from Sigma Chemical Company. Beads were Sigma LB-11, 1.091 μm particle diameter, polystyrene latex. Variations of culture media were made by mixing stock solutions of the appropriate components (Johnson et al., 1980; Soldo et al., 1966).

Macronutrient Analysis

Glycogen Assay. Glycogen concentration was determined by the iodine-iodide method as originally described by Krisman (1962) with modifications suited to this experiment. Standards ranged from 0 mg/ml to 1 mg/ml glycogen. Absorbance was measured at 460 nm on a Gilford 2600 spectrophotometer. Protein Assay. K&K ovalbumin was assayed by the standard Bradford method (1). Standards ranged from 0 mg/ml to 1 mg/ml. Absorbance was measured at at 595 nm.

RESULTS

The results of determination of glycogen and protein with culture age are presented in Figures 1 and 2. The data in Figure 1 show that the macronutrient consumption in Johnson's chemically defined medium gives the same pattern as in Soldo's crude medium as determined by Fok, Allen and Kaneshiro (1981). The glycogen is utilized extensively and the bulk of the protein remains in the culture medium. Figure 2 shows that there is a reciprocity between glycogen depletion and cell population. The simplest summary for these experiments is that Johnson's medium is a carbohydrate-based economy and parallels the results with Soldo's crudely defined medium. In each case, one macronutrient is utilized and the protein component is not completely used.

An attempt to bypass the protein requirement was made by providing Soldo's sheep-brain lipids and Johnson's mussel glycogen together. Eleven lines of axenic paramecia (*Paramecium tetraurelia*, *Paramecium sonnebornii*, *Paramecium multimicronucleatum* [5 strains from different parts of the world], *Paramecium caudatum* [2 strains], *Paramecium primaurelia*, *Paramecium octaurelia*) failed to grow on this formulation of both macronutrients together in Johnson's basal medium with small components.

Replacement of the protein with particulate matter was tried with 1.1 μm latex beads. All eleven lines failed to grow on a bead-glycogen formulation in Johnson's basal medium with small components.

An amino acid mixture which contains glutamine and cysteine, the missing two of the "magic-twenty" amino acids in the chemically defined recipe, was used to test the hypothesis that the protein overcomes the amino acid imbalance in the recipe. Again,

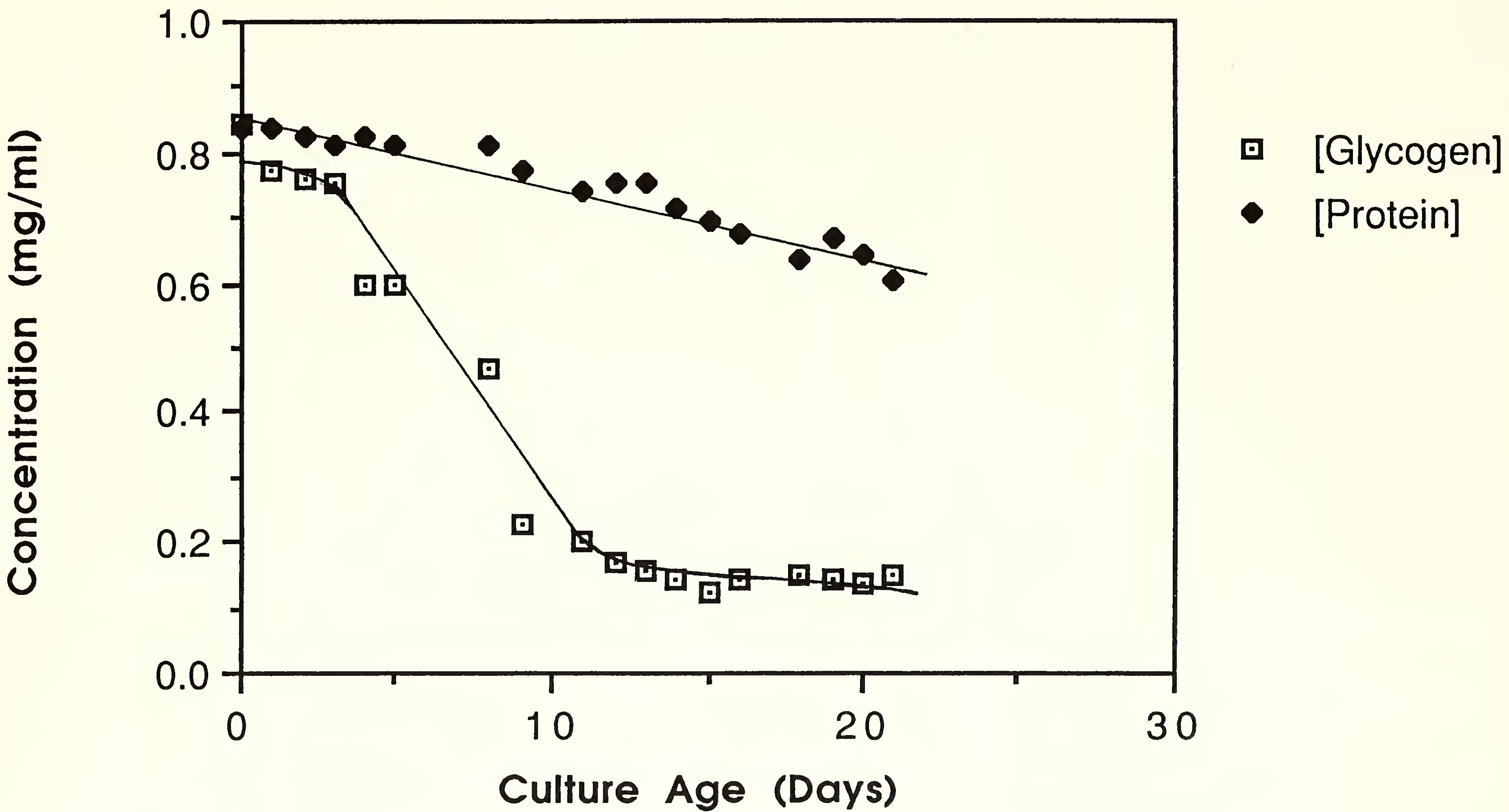

Figure 1. Depletion of glycogen and protein from the culture medium. The majority of the glycogen is removed in a sigmoid fashion, while the protein is depleted linearly, with the majority still present at the end of the growth cycle.

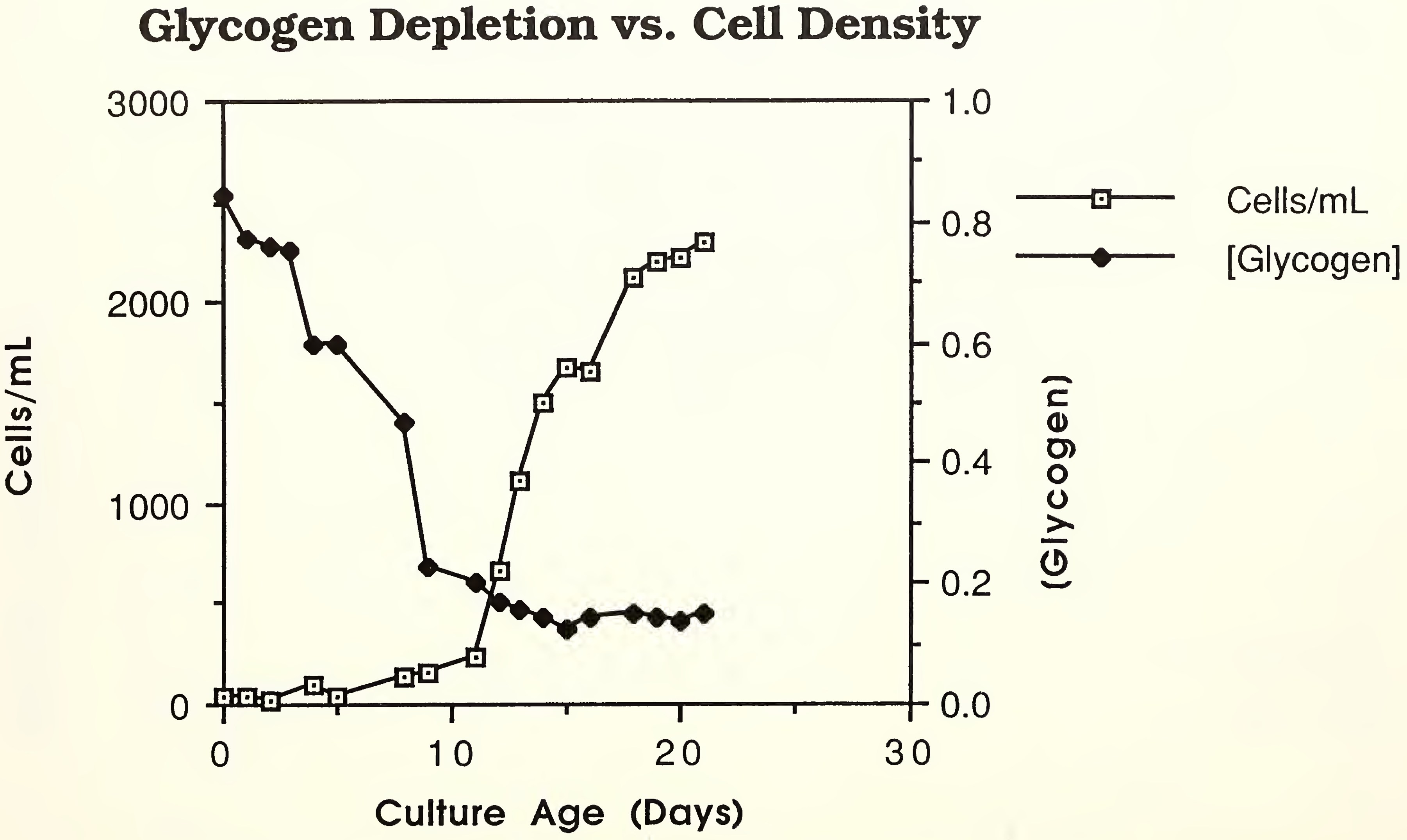

Figure 2. Change of glycogen in the medium with advancing culture age.

none of the eleven axenic lines survived one growth cycle on glycogen with all twenty amino acids present in absence of the protein source.

DISCUSSION

The chemically defined medium of Johnson is a carbohydrate-based economy, whereas the crude medium of Soldo is a lipid-based economy. In each system, one macronutrient is utilized completely and about 70-75% of the protein remains. What is the function of the protein in these axenic cultures? Even though a majority of the protein is not utilized in a growth cycle, if it is left out, growth does not occur.

In the case of Soldo's medium, the proteose-peptone or trypticase could supply needed amino acids for the cells and incomplete utilization could be related to the method of uptake: the lipid could soak into the membrane of the cell, whereas the protein would be ingested only by food vacuole formation.

In the case of Johnson's defined medium, both protein and glycogen would be taken in through endocytosis. Also, 18 of the 20 ''magic'' amino acids are provided while cysteine and glutamine are not provided. Hydrolyzed ovalbumin does not replace the protein. Thus, perhaps the protein provides glutamine, since glutamine would be converted to glutamic acid during acid hydrolysis. Glutamine is a noted stimulator for the Krebs cycle and is routinely added to tissue culture media (Freshney, 1987). The effect of added glutamine and cysteine on paramecium growth is clear; they alone, at concentrations similar to those of the other amino acids in the mixture, do not provide the necessary growth stimuli.

In both the lipid-rich and carbohydrate-rich formulations, the role of protein could be related to stimulation of food vacuole formation (Fok et al., 1988): the protein interacts with the other macronutrient to form particles which stimulate uptake or is able to do so alone. In the case of interactions, Schiff base formation between protein amino groups and aldehydes in carbohydrates would be a reasonable mechanism.

Reilly (1964) has discussed the importance of particulate matter in the medium of axenic *P. caudatum* and found that syntheic magnesium silicate would replace the protein requirement for this species group. We have not been able to repeat this work with *P. multimicroncleatum* . Fok, et al. (1988), have studied the effect of particulate matter in the form of polystyrene beads on food vacuole formation. With respect to numbers, a minimum of about 10^7 beads ml^{-1} is required for appreciable vacuole formation. Food vacuole formation is linear to 4.0×10^8 beads ml^{-1}, and further increases occur at over 6×10^{10} beads ml^{-1}. Thus, much particulate matter is required for vacuole formation; perhaps the protein component participates in this function and is not utilized as a nutrient component. If the protein's major role is to provide a mechanical stimulus for food vacuole formation, we have not come close to finding the appropriate substitute. We do know that if the complete medium is filtered with a sterilizing filter (0.22 m diameter), the growth supporting capability of the medium is lost.

This report emphasizes the role of proteins in the nutrition of axenic paramecium. Future attempts to pinpoint the role of the required protein will concentrate on both nutritional and physical-particulate mechanisms and the possibility that both play a part.

ACKNOWLEDGMENTS

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