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EFFECT OF ENERGY SOURCE ON GROWTH AND RESPIRATION OF *T. PYRIFORMIS*

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ABSTRACT

Mou-Liang Chen (1970) *Effect of Energy Source on Growth and Respiration of T. Pyriformis*. Bull. Inst. Zool., Academia Sinica 9(1): 1-5. The effect of different energy and nitrogen source on the growth and metabolism was tested in two strains of *T. pyriformis*. Greatly reduced growth rate was obtained in fructose and glutamic acid media, but no significant effect was observed on GOT, GPT and ATPase. Using different carbohydrate as energy source did not affect oxygen consumption, but slightly lower respiratory quotient was shown in fructose medium. If glutamic acid was used as the energy source, both oxygen and carbon dioxide exchange rates were markedly reduced. In case of using amino acid mixture as nitrogen source, marked reduction of carbon dioxide liberation was observed while oxygen consumption remained constant. In all cases, respiration of aged ciliates was markedly reduced.

The protein synthesis of *T. pyriformis* had been found to be 50 percent greater in the presence of dextrin than of glucose (9). It had been demonstrated that the cell composition and several enzyme activities were also affected by the medium as well as by the period of incubation (1, 2). Thus, the metabolic rate of the ciliates was not unique in different media. Further studies of the ciliate metabolism might shed new light on the relationship between growth and metabolic changes in the utilization of medium nutrients.

MATERIALS AND METHODS

The MC and W strains of *T. pyriformis* were maintained in medium containing 2.0% Bactopeptone, 0.1% yeast extract, 0.1% glucose and 0.1% sodium chloride. Two drops of 3-day cultures were used as inocula for each 100 ml of test medium. Composition of basal medium used for experiments is detailed in Table 1. Nitrogen and energy sources were supplemented at 1 mg-N/ml and 0.5% respectively. Preparation and sterilization of experimental cultures were reported elsewhere (1). At appropriate period of incubation at 25°C. 3 flasks of each test material were pooled for determinations. Cell counts were made in a hemacytometer on pooled samples inactivated with buffered formaldehyde. For enzyme activity determinations, cultures were chilled to 5°C. Ciliates were separated from medium by centrifugation and washed twice with distilled water before being homogenized with a Teflon homogenizer. The glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities of the cell-free suspension were determined by the colorimetric method of Reitman and Frankel (8), while the ATPase activity was determined by the method of Keiley (6). Respiration of ciliates was measured by Warburg's direct method (12) with 20ml. standard conical flasks.

RESULTS

During the test experiments, although

repeatedly tested by method of Swanson(11), no glucose-6-phosphatase activity could be detected. This indicates that the enzyme is absent and that ciliates are different from vertebrates.

The effect of medium energy source on the growth and cellular transaminase activites are shown in Table 2. When glucose or starch was used as the energy source, approximately equal growth rate

TABLE 1

Compsition of Basal Medium

Component	mcg/ml	Component	mcg/ml
Ca-pantothenate	0.625	$MgSO_4 \cdot 7H_2O$	140
Nicotinamide	0.625	$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	62.5
Pyidoxine•HCl	6.25	$ZnCl_2 \cdot H_2O$	0.125
Pyridoxal	0.625	$MnCl_2 \cdot 4H_2O$	30
Riboflavin	0.625	$CuCl_2 \cdot 2H_2O$	1.25
Thiamine•HCl	6.25	$FeCl_3 \cdot 6H_2O$	3
Folic acid	0.0625	K_2HPO_4	0.75
Inostitol	0.625	KH_2PO_4	175
Choline chloride	6.25	Guanine	175
PABA	0.625	Adenine sulfate	50
Biotin	0.0625	Cytosine	50
Lipoic acid	0.02	Uracil	50

TABLE 2

Effect of Medium Energy Source on Transaminase and Adenosine Triphosphatase of 3-day Cultures

Energy source	population	GOT	GPT	ATPase
	Cell/ml$\times 10^{-4}$	Units/cell$\times 10^6$		μm-P/min/cell$\times 10^6$
Strain W				
Glucose	15.7	562	92	5.3
Starch	16.9	518	80	5.5
Fructose	11.0	505	95	5.0
Glutamic acid	11.7	613	98	4.8
Strain MC				
Glucose	19.2	500	86	10.8
Starch	19.3	488	72	12.2
Fructose	10.5	535	98	10.3
Glutamic acid	10.8	587	102	11.6

was obtained, while with fructose or glutamic acid as the energy source, much slower growth rates were obtained. The GOT and GPT were, however, not significantly affected by the medium energy source. Furthermore, the slower rate of growth and absence of effect on transaminase, particularly GPT, with glutamic acid brought forth the difference between the ciliates and mammals.

TABLE 3

Effect of Medium Energy Source on Respiration of Ciliates

(μl/hr/million organisms)

Energy source	3-day			6-day		
	Q_{O_2}	Q_{CO_2}	RQ	Q_{O_2}	Q_{CO_2}	RQ
Strain W						
Glucose	144	117	0.81	132	110	0.83
Starch	149	119	0.80	133	108	0.81
Fructose	154	106	0.69	120	76	0.63
Glutamic acid	117	65	0.55	94	51	0.54
Strain MC						
Glucose	220	167	0.76	150	111	0.74
Starch	211	159	0.75	143	96	0.69
Fructose	218	146	0.79	139	82	0.59
Glutamic acid	137	90	0.66	97	61	0.63

The effect of medium on the respiration of ciliates is shown in Table 3. It can be seen that strain MC acquired a much higher rate of oxygen consumption and carbon dioxide liberation than strain W. Medium carbohydrate did not affect the oxygen consumption appreciably; however, lower rate of carbon dioxide exchange was obtained when fructose was used as the sole energy source. In the case of glutamic acid, respiration rates were considerably low. The results might indicate relatively incomplete utilization of fructose and glutamic acid by the cilates with a consequent lower rate of multiplication. Although the change of respiratory quotient showed no definite order with the period of incubation, higher values of oxygen consumption and carbon dioxide liberation were generally noted in cultures of younger age, i. e., 3-day cultures.

In comparing the effect of nitrogen source on the respiration of ciliates, organisms cultured from stock peptone medium were washed free from the medium and then resuspended in casein or amino acid medium (1 mg-N/ml). After standing for 2 hours at 25°C respiration was measured. Results showed that change of medium nitrogen source did not affect oxygen consumption, while reduced rate in carbon dioxide liberation in the amino acid medium was obrerved. The marked reduction of respiratory quotient in amino acid medium might mean a great hindrance of overall utilization of medium energy source, leading to ineffectiveness of energy trapping and biosynthesis, and slow rate of multiplication.

DISCUSSION

Glucose-6-phosphatase and transaminase

(GOT, GPT) have been known to be affected by the dietary energy source in animals (3, 4, 5). Change of dietary energy source either in quantity or in quality will alter the metabolic pathway and result in the change of related enzyme systems. The test organism used in the present experiments differed from the vertebrates which utilized carbohydrates directly after uptake from tne medium. The lack of glucose-6-phosphatase might account for the equal growth rate and transaminase activities obtained from the glucose and starch media. Waldorf *et al.* (13) reported that limited food intake can result increased liver GOT and GPT activities in rat. Although no direct evidence of similar nature had been shown in ciliates; nevertheless, in view of the many similarities in nutritional requirements and metabolic processes between the ciliates and vertebrates, the slower growth rate occurred in the fructose medium might not be attributed to the less effectiveness in uptake from medium. Since neither GOT nor GPT was affected, the slightly lower respiratory quotient might indicate that less complete utilization of fructose could be the primary factor for the slightly reduced growth rate.

It had been demonstrated in mammalians that GPT played an important role in the gluconeogenesis (10). Our previous findings (2) showed that when an amino acid mixture was used as the nitrogen source, ratarded growth was obtained. Similar results were obtained in the present study when glutamic acid was used as energy source. However, it was unable to denomstrate the effect of medium composition on both transaminase and ATPase. If the response of ciliates toward nitrogen and energy assimilation was similar to that shown in vertebrates (10, 13), then the lower respiration rate with insignificant increased GOT and GPT activities shown in glutamic acid medium could possibly suggest that, uptake rather than utilization was reduced. However, such interpretation should be appraised by further metabolic studies with labeled amino acids or carbohydrates.

TABLE 4
Respiration of Washed Ciliates (MC) in Different Protein Media
(μl/hr/million cells)

Medium	Population cells/ml	Q_{O_2}	Q_{CO_2}	RQ
Casein	100,000	140	102	0.73
	50,000	148	110	0.74
Amino acid	100,000	137	80	0.59
	50,000	160	95	0.59

It can be seen that the two strains of the test organism, similar in nutritional requirements and morphology, differed in rates of metabolic process. It should be pointed out that increased cell population could affect the rate of respiration during measurement. McCashland and Kronschhable (7) reported that respiration was varied directly with nutrient availability. Present experience showed that cell populations of less than 200,000/ml would not show significant lowering of respiration while reproducibility was satisfactory.

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