ACTION OF OXYTHIAMINE ON TETRAHYMENA GELEII W

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No pure culture of protozoa has thus far been achieved except for some euglenae, belonging to chlorophyll-containing Flagellata. The researches on general metabolism and nutritional requirement of protozoa have been left far behind those of bacteria or eumycetes. Recently, however, Kidder et al. (1–5) have succeeded in purely culturing Tetrahymena and its adjacent ciliata in a synthetic medium. The study on the metabolism of protozoa has thus become accessible to microbiologists. Many reports of Kidder et al. concerning the effect of antivitamins on Tetrahymena have already appeared, especially, the inhibitory action of neopyrithiamine on the growth of the organism and the reversal of the effect by an appropriate dose of thiamine. The authors have tried to study from another point of view the metabolism of the organism using oxythiamine, i.e., a thiamine derivative substituted with OH instead of amino group in the 4-position of the pyrimidine and to observe at the same time the morphological change of the organism with the aid of phase contrast microscope, for clarifying a part of the mode of action of thiamine.

EXPERIMENTAL

Methods

Culture Medium—Two per cent peptone solution, adjusted to pH 7 by adding 10 per cent NaOH, was autoclaved at 120°.

Culture—Ten ml each of the culture medium was placed in each flask and autoclaved at 120°. Tetrahymena was inoculated into the medium from the original culture. The cultures were incubated at 25°, the optimum temperature for the organism. Maximum growth was found to be attained within 5–7 days.

Addition of Reagent—Two different methods of adding the reagent was adopted: (a) Varying concentrations of oxythiamine were respectively added prior to inoculation of the organism. (b) The reagent was added after the organism had approached the maximum growth.
Observation—For calculating the population, an erythrocyte-counting plate and a simple dark-field method (6, 7) were adopted. For measuring body length, a micrometer was used, which was inserted in an ocular and the mean values of ten observations were noted. The morphological change was observed by a phase contrast microscope, simultaneously by taking photograph.

Results

1. The Addition of Oxythiamine prior to Inoculation.

Varying concentrations \((10^{-2}-10^{-5} \text{M})\) of oxythiamine were added respectively. The population, body length and morphological change of the organism were observed from 24 to 168 hours after inoculation.

As shown in Fig. 1, the growth of the organism was markedly inhibited by the addition of oxythiamine. Scarcely any growth was observed within 7 days after adding \(10^{-2} \text{M}\), and in lower concentrations some inhibitory effect was perceived depending on concentrations. However, no considerable change in size and shape could be observed.

2. The Addition of Oxythiamine at Various Periods of Culture.

The culture was continued without any treatment for 3 days, and when the growth of the organism approached the maximum, oxythiamine in a final concentration of \(10^{-3}-10^{-6} \text{M}\) respectively was added with the results represented in Fig. 2.

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Fig. 1

Influence of Oxythiamine on the Growth of Tetrahymena when Added prior to Inoculation.

K, control without oxythiamine.

Fig. 2

Influence of Oxythiamine on the Growth of Tetrahymena when Added after Durations of Culture.

K, control without oxythiamine.
The times in the Figure show the elapsed times after the addition of oxythiamine rather than the duration of the culture. The body length of the organism in control experiments was about the same for 24 – 240 days, averaging $50 \pm 5 \mu$. After the addition of oxythiamine, a characteristic shortening of the body length versus time was noticed, depending on the concentration of oxythiamine, i.e., in the concentration of $10^{-8} M$, the size was reduced to $1/3$ in 10 days. No specific abnormality in mobility was perceived but it became inactive with the shortening of the cell. A very noticeable change in morphology was the appearance of a huge vacuole in the caudal half of the cell. The site in which this abnormal vacuole appeared, corresponded approximately to the site of a normal contractile vacuole but the vacuole caused by oxythiamine failed to pulsate as the normal contractile one and was far larger in size. Fig. 3 is a photograph of a living cell taken with a phase contrast microscope, showing an abnormal state with a vacuole formation. The lens for the phase contrast microscope was $40 \times$ Bright High of Tiyoda, and the film for the photograph was Fuji-SS.

DISCUSSION

Many researches have hitherto been made on the metabolism of thiamine and the mode of action in yeasts, bacteria and molds as well as in mammals and fowls. It has been, however, considered so far to be very difficult to realize the role of thiamine played in a single animal cell carrying out the same metabolism as mammalian cells and requiring similar nutrients. The cytological and biochemical study on thiamine deficiency of the above-mentioned single animal cell has also remained to be a difficult problem. Since Kidder et al. it became possible to study the above subject. Above all, with the solution of the two important actions of thiamine, i.e., the problem on protogen and the relationship among protogen, thiamine and lipothiamide, Tetrahymena became one of the most important organisms for researching the biochemical and cytological behaviours of thiamine. Tetrahymena geleii $W$ was therefore made thiamine-deficient using oxythiamine, and the change of the organism was observed.

Oxythiamine is an important antivitamin in a somewhat different sense from neopyrithiamine: Neopyrithiamine is an analogue of thiamine, produced by exchanging pyridine for the thiazole moiety, the NH$_2$-group at the 4-position of the pyrimidine moiety being intact just as in thiamine, whereas in oxythiamine the NH$_2$-group at the C4-position of the pyrimidine is substituted by OH. Neopyrithiamine may act as an antivitamin, at least in
part, after being converted into lipothiamide derivative, whereas oxythia-
mime, being incapable of forming lipothiamide, has only one antivitamin
action. Therefore, the inhibition of growth and shortening in size which
*Tetrahymena* suffers in the presence of oxythiamine, may possibly be due to
an abnormal metabolism taking place in a stage prior to lipothiamide for-
mation of thiamine *in vivo* or the inhibition of thiamine action entirely
independent of lipothiamide formation.

Oxythiamine has been shown to inhibit the growth as well as the normal
development of body structure, and, in consequence, it may be assumed to
prevent the protein synthesis in *Tetrahymena*. Whether or not the inhibi-
tory action of oxythiamine depends upon the blocking of tricarboxylic acid
cycle due to the inhibition of pyruvic acid metabolism caused by thiamine
deficiency remains to be established.

The formation of an abnormal huge vacuole is assumed to be a manifes-
tation of plasm protein degradation due to protein deficiency rather than
an abnormal enlargement of a contractile vacuole.

**SUMMARY**

1. The addition of oxythiamine in final concentrations of $10^{-2}-10^{-5} M$
markedly inhibited the growth of *Tetrahymena geleii W*, only when the
vitamin derivative was added prior to the inoculation of the organism.
2. When the vitamin derivative was added in the same concentration
after the arrival of the maximum growth of the organism, the inhibitory
action was not observed, but a characteristic shortening of cell length and
an abnormal huge vacuole formation took place, resulting finally to the death
of the organism.
3. The above results seem to suggest the possible relationship between
the amount of culture medium and the amount of protein synthesized *in vivo*
by absorbing the nutrients.

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**REFERENCES**