INHIBITORY EFFECTS OF FUSARENON ON MULTIPLICATION OF TETRAHYMENA PYRIFORMIS

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SUMMARY: A toxic substance named fusarenon was isolated from rice grains infected with Fusarium nivale. The substance, at concentrations of 50 μg/ml or higher inhibited completely the division of Tetrahymena pyriformis (ciliated protozoan) in exponentially growing mass culture and in temperature-induced synchronous culture.

From the delay in cell division caused by exposure of the cells to 200 μg/ml of fusarenon for 20 min at various stages after the end of the synchronous treatment, it was shown that the cells at 30 min before the synchronous division maximum were most susceptible to the toxic substance. The cells at this stage are known to have completed the syntheses of DNA and m-RNA and be actively synthesizing the specific protein, all of which are indispensable with the forthcoming division.

From pulse-labeling with 3H-thymidine, -uridine or -phenylalanine in the presence of fusarenon at different concentrations, it can be said that fusarenon affects most seriously the protein synthesis. It is, therefore, likely that the blockage of the cellular protein synthesis caused by the toxin may result in the inhibition of cell division.

INTRODUCTION

It has been reported that severe injuries occurred occasionally in domestic animals from ingestion of grains infected with Fusarium or Gibberela. Recently, several reports have appeared dealing with the nature of toxic substances produced by members of the genus Fusarium (Gilgan, Smalley and Strong, 1966; Dawkins, 1966; Yates et al., 1968). All the toxic substances isolated by the above authors, however, are not the same. Fusarenon, a toxic substance purified by Tsunoda et al. (1968), is assumed to be a terpenoid on the basis of its chemical nature. Studies have been made on the injurious action of fusarenon on mice (Okubo and Isoda, 1967) and on tissue culture cells (Ohtsubo, Yamada and Saito, 1968).

In the present paper, the effects of fusarenon on the cell division of Tetrahymena pyriformis were further studied to analyze the mode of action of this toxic substance, since Tetrahymena cell has been extensively studied with respect to its nutritional requirements, response to chemical and physical agents and synthesis of macromolecular components in relation to its cell-life cycle.
MATERIALS AND METHODS

**Fusarenon**: Fusarenon was purified as described previously (Tsunoda et al., 1968). Because of its insolubility in water, fusarenon was dissolved in a culture medium to a desired concentration by the aid of Tween 80 at a final concentration of 0.05%.

**Cell culture**: The ciliate protozoan, *Tetrahymena pyriformis* strain W, was grown under sterile conditions in culture medium containing 2% proteose-peptone, 0.5% yeast extract and 0.87% dextrose. An inorganic medium (2.75 g NaCl and 0.25 g MgSO₄·7H₂O in 1000 ml of 5 mM phosphate buffer, pH 6.5) was used for washing the cells.

The temperature-treatment for induction of division synchrony was a series of alternate shifts between 26°C and 34°C (Scherbaum and Zeuthen, 1954). In the system, synchronous division appears at 75 min after EHT (the end of the heat-treatment), the maximum division index being nearly 90%.

Cell counts were performed by the method of Watanabe (Watanabe, 1963).

**Pulse-labeling**: The cells cultivated in the presence of various concentrations of fusarenon were pulse-labeled with 1 μCi/ml of ³H-thymidine, ³H-uridine or ³H-phenylalanine (New England Nuclear Corp.). At the end of the labeling, the cells were fixed with cold 5% trichloroacetic acid and washed to free the excess isotope. They were then collected onto 23 mm circular filter paper and further washed with 95% ethanol. After drying, the filter paper was placed onto a planchet and the radioactivity was measured by windowless gas-flow counter (Aloka).

EXPERIMENTAL RESULTS

**Effect of Fusarenon on the Cell Multiplication**

Fig. 1 represents the results of an experiment in which fusarenon to various concentrations (1, 10, 50, 100 and 200 μg/ml) was added to the exponentially growing *Tetrahymena* cultures (about 5 × 10⁴ cells/ml) in proteose-peptone medium. At 0.1 μg/ml or lower concentrations, fusarenon did not affect the cell multiplication significantly (not included in the figure). At 1 μg/ml, it delayed slightly the recommencing of the multiplication and at 10 μg/ml the effect was greater. At 50, 100 or 200 μg/ml, it completely blocked the cell multiplication. At 200 μg/ml, spherical dead cells appeared at 9 hr after the addition of fusarenon.

**Effect of Fusarenon on the Synchronous Cell Division**

Fusarenon to various concentrations was added to the temperature-induced synchronous cultures at 5 min after EHT. As shown in Table 1, at 1 μg/ml, fusarenon caused about a 30-min delay of synchronous division; at 10 μg/ml, it delayed the onset of the first synchronous division by about 240 min; at 50, 100 or 200 μg/ml, the synchronous division was completely suppressed.

**The Most Susceptible Cell Age to a Short-Time Exposure to Fusarenon**

Fig. 2 shows the delayed divisions induced by 20-min exposure to fusarenon at 200 μg/ml started at various stages after EHT. In this experiment, the synchronized cells were exposed to fusarenon for a certain period, after which the inhibitor was removed by fourtimes repeated washings in a hand centrifuge. By these processes,
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Fig. 1. Effect of fusarenon on exponentially growing Tetrahymena cells. Fusarenon was added to the medium at 0 hr at the concentrations as follows: 1 μg/ml (▲); 10 (□); 50 (■); 100 (■); 200 (×) and control (●).

Table 1. Effects of various concentrations of fusarenon on synchronous division*

<table>
<thead>
<tr>
<th>Conc. of fusarenon (μg/mg)</th>
<th>Occurrence of synchronous division**</th>
<th>Mid-point of the first synchronous division (min after EHT)</th>
<th>Maximum division index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>110</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>240</td>
<td>40</td>
</tr>
<tr>
<td>50</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>100</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>200</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

* Fusarenon was added to synchronous cultures at 5 minutes after the end of the heat-treatment (EHT).
** Observation was continued up to 300 minutes after EHT.

fusarenon was diluted more than 1,000-fold. As seen in the figure, the delay in division increases gradually during the first 45 min after EHT, followed by a sharp decrease in the sensitivity. After the transition-point (45 min) up to the synchronous division, the addition of fusarenon does not exert any harmful influence upon the oncoming division. The maximum delay was observed about 45 min after EHT, in other words, about 30 min before a mid-point in fission.

**Effect of Fusarenon on the Biopolymer Syntheses**

To elucidate the mode of inhibitory action of fusarenon, synthetic activities of DNA, RNA and protein were examined under the presence of fusarenon. The log-
Fig. 2. Delays in synchronous division induced by treating the cells with 200 µg/ml of fusarenon for 20 min at various stages. Delays of division are plotted against the age of the cells when treatment with fusarenon was initiated.

Phase cells were washed quickly with the inorganic medium and resuspended in 0.9 ml of the inorganic medium containing fusarenon at 0.2, 2, 20 or 200 µg/ml. Each series of these cultures were kept standing for 10 min at 26°C and then pulselabeled with ³H-thymidine, -uridine or -phenylalanine (each 0.1 ml of 10 µC/ml or tritiated precursor solution was added). In 10 min labeling was stopped by adding an equal volume of 10% cold trichloroacetic acid and the radioactivity in each culture was measured.

Fig. 3 shows the incorporation of precursors into nucleic acids and protein under the fusarenon-inhibition. The higher the concentration of fusarenon was the less biopolymer syntheses took place. The protein synthesis was most seriously affected by the fusarenon-treatment. The concentrations of fusarenon inducing 50% inhibition in protein, DNA and RNA syntheses were estimated to be 1.7, 20 and 300 µg/ml, respectively.

A similar tendency was observed when the log-phase cells were pre-incubated in a fusarenon solution for 30 min and then exposed to radioactive precursor for another 30 min. The concentrations for 50% inhibition in the respective biopolymer syntheses were found to lie very close to one another as compared with the 10 min–10 min system shown in Fig. 3. This would suggest that the experimental results by the 10 min–10 min system give a more accurate picture of the inhibitory action of fusarenon.
Fig. 3. Effect of fusarenon on incorporation of precursors into nucleic acids and protein. Fusarenon was added to the culture at various concentrations indicated in abscissa and incubated for 10 min. Then the cells were incubated for another 10 min with $^3$H-thymidine (▲), -uridine (●) or -phenylalanine (■). Each symbol represents the average of 2 separate experiments.

renon than those in the 30 min–30 min system, since in the latter case many indirect influences are obviously superimposed upon the early effect caused by fusarenon.

DISCUSSION

The present study showed that fusarenon at 50 μg/ml blocks the cell division of synchronized and logarithmic phase Tetrahymena pyriformis W (Fig. 1, Table 1). T. pyriformis was the most susceptible to fusarenon at 30 min before the mid-point in division (Fig. 2). This indicates that a fusarenon-sensitive process is involved in the cell division. The cells at this stage are known to have completed the duplication of DNA and the m-RNA synthesis necessary for the next division (Zeuthen, (1961); Cerroni and Zeuthen, (1962); Lazarus, Levy and Scherbaum, (1964); Moner, (1967)). The synthesis of a certain protein essential for the forthcoming division has been suggested to occur actively at this stage (Zeuthen, (1961); Rasmussen and Zeuthen, (1962); Watanabe and Ikeda, (1965) a, b, c.).

This paper demonstrated that fusarenon markedly inhibited the incorporation of amino acids into protein, whereas incorporation of thymidine into DNA and of uridine into RNA were slightly inhibited (Fig. 3).

From these results it is likely that fusarenon interferes with the cell division through an insufficient synthesis of the division-essential protein for the cell division.

The present results suggested that an earlier, if not primary, effect of fusarenon
on living cells is the inhibition of protein synthesis.

Ohtsubo et al. (1968) implied the same from experimental results using cultured mammalian cells.

Attention should be payed, however, to the possible interaction of fusarenon with the cellular structural lipids and with proteo-lipids in a further step, since fusarenon is a kind of terpenoid and Tetrahymena cells contain a pentacyclic triterpenoid alcohol, named tetrahymanol, as one of the structural lipids (Mallory, Gordon and Conner, 1963).

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REFERENCES


