EFFECT OF PHYTOHAEMAGGLUTININ PHASEOLOSAXIN ON THE LIFE CYCLE OF PARAMECIUM CAUDATUM

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Key-words: phytohaemagglutinin — Paramecium caudatum — life cycle — generation time

The phytohaemagglutinin in (PHA), isolated from the seeds of Phaseolus vulgaris has a well-known mitogenic effect upon lymphocytes in vitro (6). It is established (4) that the reaction of these cells is an answer to an unspecific stimulus. In fact, the PHA shows a biological activity on other cells, too — epithelic (7), Amoebae (3), ciliates (8), certain plants (5). According to the Zech's works (8) the PHA-P (Difco) diminishes the generation time of the ciliates Bursaria truncatella and activates the growth of Stentor coeruleus in as "stationary phase" by preserving this effect for more than one generation.

The object of the present work is to study the effect of the Bulgarian PHA-Phaseolosaxin (PHA-F) on the life cycle of the infusoria Paramecium caudatum. We investigated the effect of PHA as well on the generation time of the infusoria in a logarithmic phase of growth as on isolated animals in a stationary phase.

Material and methods

We received the PHA from the Institute for infectious and parasitic diseases — Sofia where it is produced after Tznoneva's method (2). We isolated the monoclonal cell culture of Paramecium caudatum in a nutritive medium containing hay extract and inoculated with bacterial suspension (Aerobacter aerogenes, pH 6,8—7,0). The cultivation was carried out in a thermostat at 25 °C. The generation time was determined as follows: we isolated in concave glasses one animal from a culture in a logarithmic phase of growth. Immediately after the first cell division we laid one daughter cell in fresh nutritive medium and the other one — in a medium with definite PHA concentration. We measured the time for cell division. We determined the generation time for every individual by using this direct method. The generation time was calculated also according to the formula:

$$E = \frac{c \cdot T}{\log \cdot 10\ A}, \text{ where}$$

E — generation time
A — number of individuals in a given moment
c — 0,3010 (constant of transition into log 10)
T — time interval till reaching A — number of individuals.

We studied the effect of PHA on the onset of growth in animals from stationary culture by leaving the isolated individuals for 10 days and by eliminating the divided ones. Two groups were formed from the physiologically depressed

infusoria. The controls were transferred in a fresh nutritive medium and the experimental ones — in a medium with definite PHA concentration. We noted the number of dividing cells from both groups in intervals of 12 hours.

Results and discussion

We followed up the generation time of individuals in the logarithmic phase treated with 2, 5, 10, 20, 50, 100, 200 and 500 μg/ml PHA-F to establish the optimal stimulating PHA concentration. The results are shown on table 1 where the generation time (E) is an arithmetic mean of all direct determinations and it is compared with that one of the control series (E_0). The ration E_0/E characterizes clearly the stimulating effect of the PHA-F.

Table 1

Effect of different PHA concentrations on the generation time of infusoria in a logarithmic phase of growth

PHA μ/ml	Number of determinations	Mean generation time (hours)		E_0/E P
		control (E_0)	experiment (E)	
2	45	8,72±0,32	8,99±0,40	0,97>0,5
5	46	8,31±0,38	8,06±0,31	1,03>0,5
10	43	9,05±0,29	8,38±0,47	1,08>0,5
20	51	8,27±0,37	7,00±0,42	1,18<0,05
50	52	8,64±0,41	6,31±0,35	1,37<0,001
100	45	9,12±0,32	7,18±0,37	1,27<0,001
200	40	9,50±0,29	13,01±0,42	0,73<0,001
500	40	8,92±0,40	toxic	— —

These data demonstrate that definite PHA concentrations have a well-expressed stimulating effect on the infusoria by diminishing their generation time. The optimal concentration is 50 μg/ml but the higher concentrations are toxic indeed. Some authors report similar data concerning other protozoa (1,8). The generation time of the animals treated with 50 μg/ml PHA shows that the stimulating action retains during more than 48 hours. The ratio E_0/E is mean 1,41. This fact in concordance with Zech's experiments (8) and it could be considered as a characteristic peculiarity of PHA biological action.

The table 2 illustrates the possibility of PHA to shorten the term in which the stationary culture leaves the physiological depression. It shows the number of divided cells and their percentage of the total amount.

The majority of experimental individuals divide within the interval between 36 and 48 hours. This maximum is reached in the control series within the interval between 60 and 72 hours. The whole number of treated cells divide till 120 hours while in the control group 15 individuals retain yet non-divided during this period.

The present experiments demonstrate that the PHA-F concentrations of 50 μg/ml exert a stimulating effect on the life cycle of Paramecium caudatum by shortening the generation time and diminishing the term in which the culture leaves the stationary phase. Our data support the hypothesis for the unspecific character of PHA on the treated cells.

Table 2

Effect of 50 μg/ml PHA on infusoria from stationary phase culture

Hour	Control — n=71		Experiment — n=74	
	divided	%	divided	%
12	0	0	7	9,46
24	1	1,41	4	5,41
36	4	5,63	11	14,86
48	3	4,23	26	35,14
60	9	12,68	9	12,16
72	14	19,72	9	12,16
84	10	14,08	5	6,76
96	9	12,68	1	1,35
108	3	4,23	2	2,70
120	3	4,23	—	—
Total	56	78,88	74	100,0

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ВЛИЯНИЕ ФИТОГЕМАГЛЮТИНИНА ФАЗЕОЛОСАКСИНА НА ЖИЗНЕННЫЙ ЦИКЛ PARAMECIUM CAUDATUM

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РЕЗЮМЕ

Исследовано стимулирующее действие болгарского препарата фитогемаглютинина Фазеолосаксина на жизненный цикл инфузорий. В концентрации 50 мкг/мл ФГА сокращает генерационное время индивидов в логарифмической фазе роста и укорачивает срок выхода культуры из физиологической депресии в стационарной фазе. Высокие концентрации ФГА оказывают токсичное действие на инфузории.