CYTOLOGICAL STUDY OF THE ACTION OF GRIGOR'EV-SHIGA DYSENTERY TOXIN IN TISSUE CULTURE

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Grigor'ev-Shiga dysentery exotoxin caused an increase in size of the nuclei in transplanted human amnion cells after 6-72 h, an indication of degeneration of the cells. A decrease in mitotic activity of the cells and an increase in the number of dying cells were also found.

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The mechanism of action of bacterial toxins, especially the true exotoxins, has not yet been fully explained. In this connection, in recent years an increasing number of papers have been published, devoted to the study of the action of toxins at the cellular level in tissue culture [1, 4, 9, 13-14, 16-17, 19-20]. However, almost all these workers have judged the action of toxin by its cytologic effect of tissue culture, i.e., on the basis of injury to or death of the affected cells. This method of study can reveal only the general picture of changes in the culture, and details of the processes taking place are not brought to light [6-8].

The object of the present investigation was to determine the character of changes in the cell under the influence of Grigor'ev-Shiga dysentery toxin. For this purpose, monolayer transplanted cells cultures were used.

EXPERIMENTAL METHOD

Dry dysentery toxin obtained from the Perm' Research Institute of Vaccines and Sera was prepared from Strain Grigor'ev-Shiga No. 973a (Batch No. 30); LD_{50} of this toxin for albino mice is 0.09 mg.

After determination of the sensitivity of various strains of transplantable epithelial cells to this toxin cells of strain F1 (transplantable human amnion epithelial cells) were selected for further cytologic investigation. TCD_{50} for these cells is $10^{-4.71}$. TCD_{50} was taken to mean the dose of toxin causing death of 50% of the cells within 72 h after administration. The specificity of action of the toxin on the cell culture was confirmed by the neutralization reaction with homologous antitoxic serum.

The culture was grown in the usual manner on cover slips immersed in tubes containing medium. Cells cultivated for two days, with a well-formed monolayer, were used in the experiments. At certain times after addition of the toxin (3, 6, 12, 24, 48, 72 h) the cover slips were removed at the same time as the controls, fixed with Bouin's mixture, and stained with hematoxylin-eosin.

The area of the nuclei was measured by Vermel's method [2, 3]. The technique of measurement was the same in all cases: objective 96 ×, ocular micrometer 8 ×. In each case at least 100 nuclei with no marked structural changes were measured. The frequency of the various states of the nuclei (mitosis, amitosis, multinuclear and pycnotic cells) was expressed as their number per thousand cells in different parts of the specimen. In each specimen at least 2000 cells were counted. Altogether 15 series of experiments were performed with different doses of toxin (TCD_{25}, TCD_{50}, TCD_{75}, TCD_{90}).

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TABLE 1. Changes in Mean Area of Nuclei following Action of TCD_{50} of Toxin

Time after infection (in h)	Control	Expt.	t	Increase (in percent)
	$M \pm m$			
3	30,966±0,331	30,259±0,286		
6	28,518±0,315	30,301±0,348	3,8	6
24	28,833±0,302	36,066±0,498	12,5	25
72	32,322±0,466	36,733±0,782	4,8	13,5

TABLE 2. Number of Mitoses and Pycnotic Cells (per thousand cells) under Normal Conditions and during Action of Toxin

Dose of toxin	Time after administration (in h)	Control		Expt.	
		mitoses	pychotic cells	mitoses	pycnotic cells
TCD_{25}	3	43	20	31,5	20
	6	55,5	20	40,7	42
TCD_{90}	6	55	17	No. mitoses	75% of dead cells

EXPERIMENTAL RESULTS

Examination of the specimens revealed an increase in size of the nuclei in the experimental culture compared with the control.

Changes in the volume of the nuclei in tissue culture were first described by Bucher [18], who studied nuclei after addition of chemical substances to the medium or after exposure to various physical factors. He showed that during intensification of tissue culture growth, the cell nuclei and nucleoli increased somewhat in size, while when growth of the cultures is inhibited, on the other hand, the nuclei are reduced in size. Khesin [12] described an increase in size of the nuclei following infection of a sensitive culture with various viruses, in which case the enlargement of the nuclei was associated with death of the infected cells and not with intensification of their growth. He called this phenomenon "disintegrative swelling" of the nuclei.

The increase in size of the nuclei now observed under the action of dysentery toxin occurred sooner and was more marked when higher concentrations of toxin were used. This difference was statistically significant relative to the control. Under the influence of TCD_{25} of toxin, for example, a statistically significant increase in size of the nuclei was found 24 h after exposure, reaching a maximum after 72 h (an increase of 12%). During the action of TCD_{50} of toxin, the nuclei were enlarged 6 h after exposure, reaching a maximum after 24 h, followed by a slight decrease in size (Table 1). When TCD_{75} was used, the greatest increase in area of the nuclei in the experimental specimen compared with the control was observed after 6 h (28.5%), and later in the experiment the nuclei became smaller.

These results are in agreement with those obtained by Tarusov [10], who used a method of striction and found an increase in volume of the cells (organs of recently killed animals) under the influence of both endotoxins (dysentery) and exotoxins (tetanus, diphtheria). He found that the cell volume increased under the influence of the toxin after a definite latent period, and that a strict quantitative relationship exists between the concentration of toxin and the period of incubation. According to Tarusov, the extent of the swelling also depended on the concentration of toxin.

The increase in size of the nuclei under the influence of virus [12] is independent of the dose of virus, it is clearly observable 1-2 h after infection, and thereafter it does not increase.

The causes of swelling of the nucleus (intake of water) were not completely elucidated. Most workers associate this with changes in metabolic processes leading to an increase in the number of protein molecules in the karyoplasm, as a result of which the osmotic pressure inside the nucleus is increased and so also is its volume. Some investigators [10] attribute the swelling of cells under the influence of toxins not to an increase in osmotic pressure in the cells, but to depolymerization of the cytoplasmic proteins, to a consequent increase in the surface area of the cell, and to an increase in the quantity of bound water.

What was responsible for the swelling in the present case? Was this swelling functional, as suggested by Bucher, or "disintegrative" ?

Parallel with the karyometric investigation a study was made of the frequency of occurrence of different states of the nuclei: mitosis, amitosis, multinuclear and pycnotic cells. This showed that under the influence of exotoxin, even in a dose so small as TCD_{25}, starting 3 h from the moment of administration the mitotic activity of the cells was definitely inhibited, and the number of degenerating cells was increased (Table 2). This was thus a case of "disintegrative swelling" of the nuclei as described by Khesin.

The problem of changes in mitotic activity under the influence of bacterial toxins has received inadequate attention in the literature. As a rule investigations in this direction have been carried out at the macroorganism level. Those workers [8, 11, 15] who have studied the effect of various toxins on the corneal epithelium of mice have shown that it is antimitotic in character. It was interesting to study the changes in mitotic activity under the influence of exotoxins in an isolated cell system.

A statistically significant decrease was found in the number of mitoses starting from 3 h after administration of toxin in doses of TCD_{25} and TCD_{50}. With doses of TCD_{75} and more, only solitary mitoses were found after 6 h, or they were completely absent (Table 2).

No significant differences could be found in the relative proportion of multinuclear cells or amitoses under normal conditions and after administration of toxin.

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