SENSITIVITY OF SOME TISSUE CULTURES TO THE TOXIN OF *Clostridium sordellii*

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UDC 576.851.555.097.29.093.35

Of 19 tissue cultures studied, that from 11-day chick embryos was most sensitive to the action of the toxin of *Clostridium sordellii*.

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Investigations have been made [1, 2, 4] of the cytotoxic action of the toxins of *Clostridium histolyticum*, *Cl. oedematiens*, *Cl. perfringens*, and *Cl. septicum* on transplanted human liver cells, monkey kidney cells, HeLa, Detroit-6, HLS, and Cynomolgus monkey heart cells and human and chick embryonic fibroblasts, and the presence of cytotoxin changes has been demonstrated in tissue cultures under the influence of these toxins.

No data could be found in the literature on the cytotoxic action of the toxins of *Cl. sordellii* on tissue cultures.

A previous study to determine whether tissue cultures can be used to titrate the toxins of gas gangrene organisms showed that the toxin of *Cl. sordellii*, like those of *Cl. histolyticum*, *Cl. oedematiens*, *Cl. perfringens*, and *Cl. septicum*, causes death of the cells in tissue cultures of human and chick embryos and transplanted Hep-2 and human amnion cells (line LF) [3].

The object of the present investigation was to study the cytotoxic action of the toxin of *Cl. sordellii* on various tissue cultures in order to choose the most sensitive tissue culture for titration of the toxin in vitro.

EXPERIMENTAL METHOD

Three types of preliminarily trypsinized tissue cultures and 16 transplanted heteroploid lines were tested in the experiment.

Preliminarily trypsinized tissues of chick embryos were cultured in Hanks' solution with the addition of 7% bovine serum, human embryonic tissues and monkey kidney cells were cultured in a 0.5% solution of lactalbumin hydrolysate with 5% of the same serum, and cultures of transplanted heteroploid lines in medium No. 199 with the addition of 10% bovine serum.

Toxin of *Cl. sordellii* (batch 4A, working dose 0.23 mg/ml, 0.0005 MLD) was prepared on March 18, 1959 in the Department of Microbiology and Immunology of Wound Infections of the N. F. Gamaleya Institute of Epidemiology and Microbiology. A weighed sample of dry toxin was dissolved in nutrient medium No. 199 in a concentration of 30 mg/ml. This dilution was used as the original material for preparing subsequent tenfold dilutions of toxin.

With observance of the rules of asepsis, the culture medium was poured from the test tubes chosen for the experiment containing a continuous layer of cells and replaced by addition of 1 ml of the test dilutions of toxin per tube. The experimental tubes, together with control tubes not containing toxin, were replaced in the incubator at 37°. Observations were kept on the cultures until the 7th-12th day, daily inspections being made under the low power of the microscope (×80).

EXPERIMENTAL RESULTS

The experiments showed that all the tested tissue cultures are sensitive to the toxin of *Cl. sordellii*, although the degree of sensitivity varied (Table 1). Changes became nearly visible after 16-20 h, and

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Translated from Byulleten' Éksperimental'noi Biologii i Meditisiny, Vol. 68, No. 7, pp. 64-65, July, 1969. Original article submitted August 27, 1968.

TABLE 1. Sensitivity of Various Tissue Cultures to the Toxin of Cl. Sordellii

Concentration of toxin (in mg/ml)	Tissue culture																		
	Preliminary trypsinized			Transplanted															
	Chick embryos	Human embryos	Monkey kidneys	Cynomolgus monkey heart	Detroit-6	HeLa	PEK	L	KB	Chang	C-18	Amnion-1	PKB	KEM	Hep-3	Amnion-LF	HK	MYO	PaO
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.3	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+
0.03	+	+	−	−	+	−	−	+	−	−	−	+	−	−	+	−	+	−	−
0.003	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
0.0003	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Note: + cytotoxin changes in cells; − no changes in cells.

increased in intensity during the next 2-3 days. The affected cells became round, after which all or some of them, depending on the concentration of toxin, fell from the walls of the tubes. As Table 1 shows, preliminarily trypsinized chick embryonic cells were most sensitive to the toxin of Cl. sordellii (concentration of toxin 0.003 mg/ml), and the cells of the other tissue cultures were less sensitive.

If the toxin was first heated to 100° for 15 min, it caused no changes in the tissue cultures.

LITERATURE CITED

1. E. M. Zemskov, Zh. Mikrobiol., No. 1, 69 (1963).
2. R. A. Saltykov, E. M. Zemskov, and V. N. Milyutin, Byull. Éksperim. Biol. i Med., No. 12, 43 (1961).
3. S. A. Shamraeva, Zh. Mikrobiol., No. 1, 117 (1965).
4. G. Penso and G. Vicary, R. C. 1st Sup. Sanitá, 20, 655 (1957).