SENSITIVITY OF TISSUE CULTURES OF VARIOUS SPECIES TO TOXIN OF Clostridium oedematiens

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A study of the action of the toxin of Clostridium oedematiens on 16 types of tissue cultures showed that cultures of human and chick embryonic tissues are most sensitive. The sensitivity of cultures of chick embryonic tissues varied with age, 11-day chick embryos being most convenient for use.

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Many data have been reported indicating that certain toxins can destroy the cells of particular tissue cultures. Many of these investigations were concerned with the action of toxins of microorganisms of the gas gangrene group [1-5].

The object of the present investigation was to study a wider spectrum of tissue cultures in order to choose the tissue most sensitive to action of the toxin.

EXPERIMENTAL METHOD

Primarily trypsinized tissues of three species (chick embryos aged from 2-4 h to 18 days of incubation, human embryos aged from 8 to 14 weeks obtained by curettage of the uterus after induced abortion, monkeys' kidneys) from 13 types of transplantable strains: Sots, Detroit-6, HeLa, PEK, KV, Chang, C-18, A-1, PKV, KEM, Hep-2, and A-LF were used in the experiments.

The method of cultivation was a slight modification of the method of monolayer cultures of trypsinized cells suggested by Enders, and the method of explants. Dried toxin of Cl. oedematiens (Batch 230; M.L.D. 0.004 mg/ml; L_t 0.2 mg/ml on March 26, 1959). The main dilution of toxin was obtained by dissolving a weighed sample in medium No. 199 to a concentration of 20 mg/ml. All subsequent dilutions were made from this. For each dilution 2-4 tubes of 24 h culture were used and each experiment was repeated from 5 to 15 times. The cytotoxic action of the toxin was assessed after 24 h under low power of the microscope (10×8).

EXPERIMENTAL RESULTS

Sensitivity of the cultures of different tissues to the action of toxin of Cl. oedematiens was first studied. The results of comparative titration showed that the tissues used in the experiment differed in their sensitivity to the toxin. In high concentrations of toxin a cytotoxic effect was observed on all cultures used. In concentrations of 0.02 and 0.002 mg/ml, only primarily trypsinized cultures of chick and human embryos were sensitive. Tissue of human embryos was less conveniently obtained for extensive practical use. Accordingly, it is preferable to use chick embryonic tissue for titration.

Since the culture of chick embryonic tissue was the most convenient object for practical use, we decided to investigate whether the sensitivity of the tissue to action of the toxin varies with age. The results of titration of Cl. oedematiens toxin on tissue cultures of chick embryos of different ages showed that tissue of embryos aged between 2-4 h and 3 days of incubation is resistant to the action of the toxin. Tissue of embryos aged 3-6 days of incubation were more sensitive to the toxin and, finally, the culture obtained from embryos at 7-18 days of incubation possessed the highest sensitivity. Microscopically the cytotoxic action was shown by cessation of growth of the cell monolayer, rounding of the cells and their separation from one another, and detachment of the cells from the tube walls (Figs. 1 and 2).

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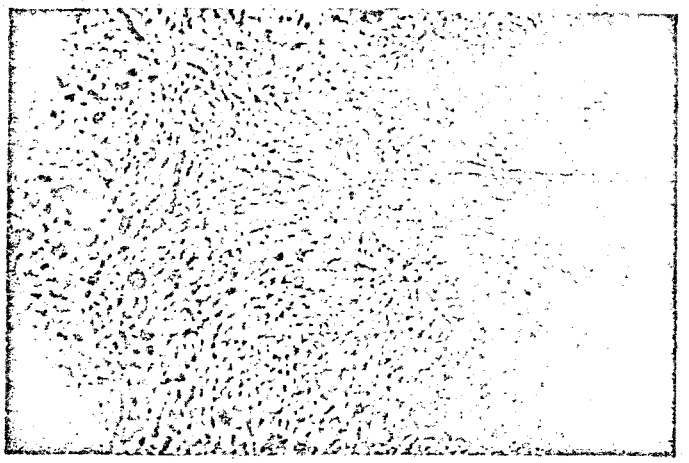

Fig. 1. 24 h Culture of tissue from an 11-day chick embryo. Control. 40 × 8.

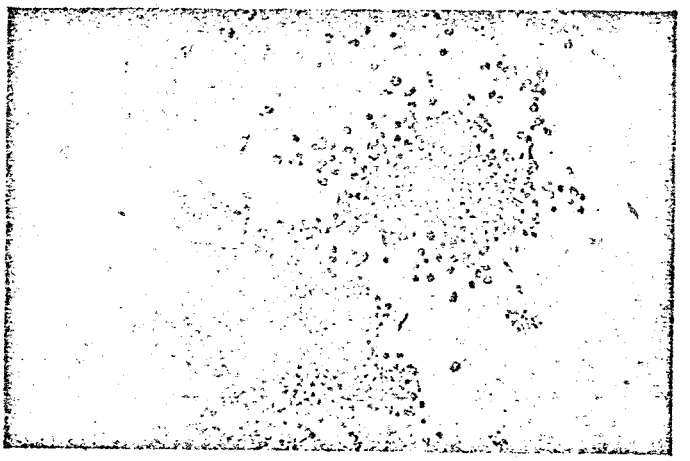

Fig. 2. Action of toxin of Cl. oedematiens in dose of 0.002 mg/ml on 24 h culture of tissue from an 11-day chick embryo. 40 × 8.

The low sensitivity of cultures of chick embryonic tissues from between 2-4 h and 6 days of incubation may be explained either by the presence of undifferentiated epithelioid cells or by incomplete development of connective-tissue cells on which the toxin acts. The increase in sensitivity with increase in age of the embryo is probably due to the fact that fibroblasts are more mature in character and become more sensitive to the action of the toxin.

When choosing a tissue for work not only its sensitivity, but also liberation of its cells on trypsinization and capacity for rapid growth must be taken into account. Embryos under 6 days of age are unsuitable for extensive practical use because the yield of cells on trypsinization is small and they have low sensitivity to the toxin. In addition, when the tissues of such embryos are cultivated, difficulties arise as regards both the technique of cultivation and assessment of the results of the reaction. The yield of cells increases with an increase in age of the embryos from 7 to 11-12 days, and then gradually decreases until 18 days. The maximal cell yield is observed in embryos at 11-12 days of incubation. Because of the presence of hair on the 12-day embryos, interfering with normal growth of the culture, they are difficult to use for practical purposes. In addition, tissue from embryos at 12-18 days of incubation gives a continuous layer only after 4-6 days of cultivation. Hence, it can be concluded from the above description that embryos at 11 days of incubation are the most suitable objects for practical use because they are highly sensitive to the action of the toxin, they give the highest yield of cells on trypsinization, and they form a continuous monolayer of tissue suitable for titration of the toxin, giving results which can be read after cultivation for 24 h in an incubator.

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