# Some Biological Characteristics of Holothurin. (19882)

ROSS NIGRELLI AND PAUL A. ZAHL.*

*From the New York Zoological Society and the Haskins Laboratories, New York City.*

Late in 1951, while working at the Lerner Marine Laboratory on Bimini, Bahamas, B. W. I., the second-named author undertook to survey a number of marine organisms with the view of determining the general toxicity as well as any possible tumor-inhibiting potency of aqueous extracts derived from their tissues. To determine their systemic toxicity the extracts of macerated whole bodies were assayed in terms of the minimum amount, parenterally injected, required to kill a 20 g white Swiss mouse. Among representatives of some 50 different marine genera so sampled was the sea cucumber, *Actinopya agassizi;* a crude water extract made from the whole body was found to be lethal to mice in very small amounts, but the exact minimum lethal dose was not determined at the time, nor was any effort made to identify the tissue responsible for the toxic factor. Working independently in 1952, and also at the Lerner Marine Laboratory, the first-named author observed that a desiccated preparation of the Organ of Cuvier of the sea cucumber contained a factor which was highly toxic against mice, certain amphibia, and fish. Nigrelli designated this water soluble factor as *holothurin,* and has described its preparation and its general characteristics(1).

The present report deals with the toxicity of this material to several protozoa grown in synthetic liquid culture, and to suspensions of tumor cells.

*Effect on protozoa.* A water solution of the desiccated holothurin material shows no loss in toxic potency when autoclaved for 30 minutes at 120°C. It may therefore be added directly to the nutrient medium of a microbiological assay organism before sterilization and inoculation. Using methods described by Hutner and coworkers(2), the holothurin material was assayed in various minute concentrations for its effect on the growth of three different protozoa: *Tetrahymena pyriformis, Euglena gracilis,* and the freshwater chrysomonad *Ochromonas malhamensis.* The results of these tests are presented in Table I. It is seen that 2.2 mg of the holothurin material per 100 ml of nutrient liquid is sufficient to stop all growth of *Tetrahymena* and *Ochromonas. Euglena* tolerates a somewhat higher concentration, but it too is ultimately poisoned to complete inhibition by the factor.

TABLE I. Effect of Holothurin on Growth of Various Assay Microorganisms.

| Level of holothurin in medium* | Growth† | | | |
|---|---|---|---|---|
| | Ochromonas, pH 5.2 | Euglena, pH 3.5 | Euglena, pH 6.5 | Tetrahymena, pH 6.6 |
| — | 6 | 2.1 | .90 | 4+ |
| .0001 | 7 | 2.7 | — | 4+ |
| .0003 | 6 | 2 | .90 | 4+ |
| .001 | 5.6 | 2 | .95 | 4+ |
| .003 | 7.3 | 2 | 1 | 3+ |
| .01 | 8.6 | 2 | .90 | 3+ |
| .03 | 7.5 | 2.2 | .90 | + |
| .1 | .0 | 2.2 | .86 | 0 |
| .3 | .0 | 1.8 | .22 | 0 |
| 1 | .0 | .52 | .22 | 0 |
| 3 | .0 | .30 | — | 0 |

* Level—ml of a 22 mg/ml water solution of holothurin, made up to 100 ml of nutrient medium.

† Growth—after 6 days incubation at 25°C, read either in photodensity units or (for *Tetrahymena*) estimated. +=trace growth; 4+=profuse growth.

*Effect on tumor cells.* Using methods described earlier(3), uniform suspensions (ten million tumor cells per ml) were prepared from 7-day-old implants of mouse Sarcoma 180. Two ml volumes of such a suspension were pipetted into 15 ml tapered centrifuge tubes. Amounts, ranging from 1$\mu$g to 1 mg, were added to the 2 ml cell suspensions. The tubes were rotated in a roller apparatus (4) at 34°C for one hour, at the end of which time 0.2 ml volumes (each containing 2 x $10^6$ tumor cells) were drawn out of the tubes and

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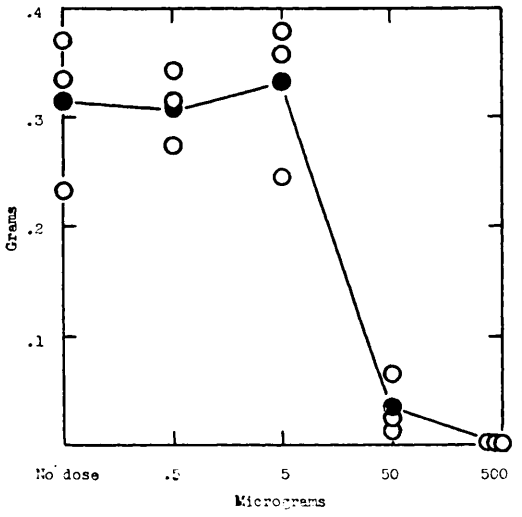


FIG. 1. Growth of tumor cell inocula pre-treated in various concentrations of holothurin. Horizontal axis indicates concentrations of holothurin-containing preparation per ml of tumor cell suspension; vertical axis indicates wet wt of tumors at the end of 7 days. Each open point on the graph represents the mean of one 5-animal experiment; solid points represent means of the three experiments. Scatter of individual wt determinations omitted because it was not dissimilar to that described in(3).

inoculated subcutaneously into mice in the usual site for axillary implantation.

At the end of a 7-day period, the growths resulting from the cell inocula were dissected out and wet-weighed. Five mice were used for each assay, and each experiment was repeated 3 times. White Swiss male mice were used throughout the experiment.

Fig. 1 indicates the effect of this treatment on the growth potency of the cell suspensions.

*Comment.* From a comparison of the data of Table I and Fig. 1, it is seen that the effect of holothurin, both on protozoa and on suspended tumor cells, is one with an abrupt threshold. The dosage difference between "no effect" and "complete killing" is very small. The significance of this is as yet not known, although the presence in holothurin of multiple toxic factors may be indicated. The toxicity of holothurin to protozoa in synthetic media is lessened by the addition of complex natural materials, such as mixtures of nucleic acids and protein hydrolysates(5). Identification of the effective antagonists in these mixtures may provide a clue to the mechanism of holothurin toxicity.

Inasmuch as very few thermostable natural organic materials have been described which are highly toxic to animals and also to protozoa and suspended tumor cells, further investigation of the active toxic principle of the sea cucumber would seem to be warranted.

*Summary.* *Holothurin*, a thermostable toxic factor derived from the Organ of Cuvier of the Caribbean sea cucumber, acts as a powerful poison to several protozoa grown in pure culture. The same material applied *in vitro* to cells of mouse Sarcoma 180 markedly reduces their growth potency.

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