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The Effects of Holothurin on Fish, and Mice with Sarcoma 180

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EMERSON & Taft (1945) have reviewed the literature on the pharmacological activity of substances derived from marine organisms. There is very little information, however, on the possible pharmacodynamic action of poisons from invertebrates (Taft, 1945).

It has been found that the sea-cucumber, *Actinopyga agassizi* Selenka, from the Bahama Banks off Bimini[1], produces a toxic agent which, in very high aqueous dilutions, will kill fish and other invertebrates. This preliminary note deals with the effects of this material (for which the name "Holothurin" is proposed) on fish, and on mice with Crocker mouse sarcoma 180.

In earlier studies it was found that sea water suspensions of the various organs of the sea-cucumber were toxic to fish in general. This toxic material was more powerful in the fluid which was emitted during autotomy. Further analysis of the organs showed that the "pink gland" (tentatively identified as the Organ of Cuvier) contained the active toxic agent. This substance was used in the tests reported here.

Twenty grams (wet weight) of the "pink gland" were suspended in 50 ml. of sea water in Erhlenmeyer flasks and used as a stock solution. Some of these suspensions were sterilized by autoclaving. The test animals were as follows: *Cyprinodon baconi* Breder, a hardy killifish from the mangrove regions of South Bimini; *Carapus bermudensis* (?), the pearl fish which normally lives in the cloaca and within the respiratory tree of the sea-cucumber; and female Swiss-Webster mice trocared in the right axillary region with sarcoma 180[2].

The test fish were placed in large stacking bowls containing two liters of sea water, to which different amounts of stock solution were added. All experiments were carried out at room temperature (30° C.).

A dilution of 1:100,000 of the stock solution killed *Cyprinodon baconi* in 23 minutes. The time necessary to kill this hardy species varied with the dilution. A dilution of 1:1,000 killed pearl fish in eight minutes and 1:1,000,000 was lethal to this species after several hours.

The mice were given subcutaneous injections of the sterilized material at the site of the tumor growth three days after implantation. One-half ml. of the stock solution killed a mouse in 2½ hrs.; 0.25 ml. killed other mice in 3 and 4 hrs., respectively. The mice were able to survive a dose of 0.05 ml. of the stock solution and of 0.25 ml. of the stock solution diluted 1:25 and 1:50 with sterile sea water. The surviving mice were sacrificed on the sixth day of tumor growth.

Histological sections of the gills of the treated fish showed that death was due to a breakdown of the capillaries. Autopsies on the mice showed massive hemorrhages in the region of the injection. In the mice which received the non-lethal doses, the sarcoma was considerably reduced in size and was necrotic. Histological sections are being prepared for a more detailed report to be published later, together with other pertinent data.

Holothurin cannot be extracted with acetone, ether, chloroform or with higher ethanol; neither is it inactivated by these solvents, by 10% formalin, 5N Hcl or 5N NaOH. The material apparently is water soluble, non-volatile and heat stable. Further, it decolorizes Lugol's solution but not methylene blue.

Additional studies on the biochemical and antitumorous properties of Holothurin, as well as of other products from invertebrates, are

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[2] The mice were supplied to the Lerner Marine Laboratory by the Sloan-Kettering Institute, New York City.

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