

SHORT COMMUNICATION

THE ORIGIN OF ANTITUMOUR ACTIVITY IN SEA ANEMONES

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Abstract—1. Crude extracts of the actinians *Radianthus papillosa* and *Anthopleura elegantissima*, both of which normally harbour symbiotic intracellular algae, show activity against ascitic tumours of mice.

2. Extracts of algae-free specimens of both species demonstrate activity equal to or greater than those of normal animals.

3. It is therefore likely that the activity originates in the animal rather than the plant material.

INTRODUCTION

SEVERAL species of Coelenterata have been shown to possess antineoplastic activity in mice (Pettit *et al.*, 1970; Sigel *et al.*, 1970; Tabrah *et al.*, 1972). Since many coelenterates contain zooxanthellae (Hyman, 1940), it has been suggested that these algae may play a role in the formation of, or indeed be the source of, pharmacologically active principles (Cieresko *et al.*, 1960). The research reported here was directed towards determining whether the compound(s) responsible for the antitumour activity of two actinian species originates with the animals themselves or in their intracellular symbiotic algae.

MATERIALS AND METHODS

The sea anemones used were *Radianthus papillosa* (Stoichactidae) from Kaneohe Bay, Oahu, Hawaii, and *Anthopleura elegantissima* (Actiniidae) from Point Alones, Monterey, California. Extracts of both species had previously demonstrated antitumour activity in mice in our laboratory, and both contain zooxanthellae. Specimens of *Radianthus* were rid of most of their symbionts by keeping them without food in an aquarium in an air-conditioned, fluorescent-lighted laboratory for a month. Individuals collected from the same locality at the same time were extracted immediately to serve as normal controls. Naturally occurring aposymbiotic (algae-free and white in colour) *Anthopleura* were collected beneath a cannery, and normal, green, algae-containing individuals were taken from nearby as controls.

Crude extracts for bioassay were made by homogenizing the anemones in five to ten times their volume of 30% ethanol, allowing the homogenate to stand at room temperature for 24 hr, centrifuging the material, filtering

and flash evaporating the supernatant to a volume in milliliters equal to the original blotted wet weight of the sample in grams, then lyophilizing it. This material was stored at -20°C until used.

The procedure used for bioassay against Ehrlich ascites tumour has been described by Tabrah *et al.* (1970, 1972). For tests against P-388 lymphocytic leukemia, female BDF₁ mice (Simonsen Laboratories, Gilroy, California), weighing 20–22 g, were injected intraperitoneally with 10⁶ tumour cells (in 0.1 ml ascitic fluid freshly drawn from mice that had been inoculated 7 days previously, diluted with Hank's solution). Extracts of *R. papillosa*, tested against both Ehrlich and P-388, were given in 0.1 ml doses, injected intraperitoneally twice a day for 10 days. *A. elegantissima* extracts were tested against P-388 only, with 0.1 ml of test solution given intraperitoneally once a day for 10 days. When higher concentrations produced chronic toxicity, alternate injections were skipped. Five mice were used for most experiments.

Results from the Ehrlich system are expressed as a percentage of non-ascitic mice alive after 30 days (some mice with solid tumours survived 1 month). Results from the P-388 system (in which all mice died within 20 days) are expressed as the average length of life of experimental mice as a percentage of that of the controls (for a particular experiment, since average control lifespan varied from 9.2 to 11.0, with an overall average of 10.3 days). The U.S. National Cancer Institute considers results above 125 per cent as indicating control of P-388.

RESULTS AND DISCUSSION

Dose-response curves of extracts of normal and aposymbiotic *R. papillosa* against Ehrlich ascites are shown in Fig. 1. Cause of death at higher concentrations was attributable mainly to toxicity, so the algae-free extracts were tested only at lower concentrations. Ascites was the major cause of death at

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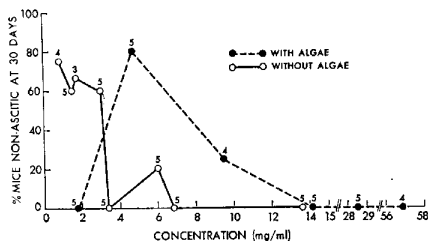


Fig. 1. Dose-response curves for crude extracts of *R. papillosa* with and without zooxanthellae (assayed against Ehrlich ascites). The number of mice tested is indicated beside each point. Concentration is milligrams lyophilized extract dissolved in a milliliter of water.

Table 1. Results of bioassays against P-388

Test solution	Concentration (mg/ml)	Mean survival time as % of controls	No. of mice	Toxicity deaths
<i>R. papillosa</i> with algae	4.7	115	5	0
	1.8	130	5	0
Algae-free <i>R. papillosa</i>	1.5	139	5	0
	0.9	112	4	0
<i>A. elegantissima</i> with algae	40.0	—	5	5
	6.7	109	5	3
	3.3	135	5	0
Algae-free <i>A. elegantissima</i>	40.0	—	5	5
	5.5	120	5	4
	2.8	133	5	1

the lower concentrations. Results of bioassays on P-388 are shown in Table 1.

The data from the three series of experiments demonstrate that the antitumour effect is present in all extracts tested. In fact, it appears that the algae-free extract has a higher specific activity than the extract from normal *R. papillosa* in the Ehrlich system. This difference is probably due to the higher proportion of animal tissue in the aposymbiotic extract. That is, the compounds of algal origin dilute the molecule(s) responsible for the antitumour effect in the extracts of normal animals. (Although some algal cells remained in the "algae-free" *Radianthus*, as revealed by tentacle smears just before extraction, if the algae were the source of the antineoplastic principle(s) the effective dose of the extract should be very much higher than that from normal animals.) It is possible that the algae had synthesized a compound which remained in the animal tissue even after the zooxanthellae themselves had been expelled, but the higher specific activity of the algae-free extract makes this unlikely. Aposymbiotic specimens of *A. elegantissima* had never possessed symbiotic algae, but show activity equal to that of normal individuals. Therefore it appears certain that in this species the antineoplastic principle(s)

originate with the animal. No "dilution" effect is apparent in the P-388 system.

Toxicity might be expected to be less with algae-free than with algae-containing extracts because zooxanthellae are dinoflagellates, some species of which are the source of saxitoxin, which is extremely toxic to mice (Kao, 1972). In fact, at equal concentrations, extracts from aposymbiotic *Anthopleura* appear to be slightly more toxic than extracts from normal animals, and the same is true of *R. papillosa* in the Ehrlich system (all bioassays with this species against P-388 were at subtoxic concentrations). These findings imply that the toxicity is also derived from the animal tissue and is "diluted" in the animal-algae extracts.

The trends are consistent despite the small number of mice tested at each dose level, and the results, using two different tumours, suggest that the antitumour activity of these two species of sea anemones, belonging to different families and from quite different environments, originates in the animal tissue rather than the symbiotic algae. However, similar experiments using extracts of cultured algal cells should also be performed. Identification of the antineoplastic principle(s) itself and determination of

the site of its production would eventually be the most definitive method of solving this problem.

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- Key Word Index*—Antitumour substance; actinians; *Anthopleura elegantissima*; *Radianthus papillosa*; aposymbiotic; ascites tumour; P-388 lymphocytic leukemia; zooxanthellae.