tion into 60-mm petri dishes. When the cultures had reached confluence, they were washed in saline and the cells were gently scraped from the petri dishes into 0.4 ml of 0.01*M* acetate buffer at *p*H 4.5. The resultant cell suspensions were broken up by ultrasonic vibration. Without prior centrifugation the sonicates were assayed for α-galactosidase, as described for leukocytes. As experimental control, the activity of β-galactosidase was measured (*11*). Protein content was determined in duplicate samples using the Folin-Ciocalteau reagent (*16*).

Fibroblasts from the affected boy showed low α-galactosidase activity in comparison to controls (Table 1). As in leukocytes, the α-galactosidase activity in uncloned fibroblasts of the proband's sister was below the range of the controls, whereas those of the mother had activity within the normal range, so that she could not have been identified as a carrier solely on the basis of these values. This is not surprising since carriers of X-linked diseases often show a wide range of phenotypic variation, which has also been observed in regard to the clinical manifestations in heterozygotes for Fabry's disease (*17*).

A total of 48 clones were isolated from the fibroblast cultures of the proband's mother and sister. Two populations of clones with respect to α-galactosidase activity were found in each woman: those with values similar to the ones found in the proband's fibroblasts (negative clones) as well as those with significantly higher activity (positive clones) (Table 1). On the other hand, both women showed only one cell population with respect to β-galactosidase. These results demonstrate the inactivation of the α-galactosidase locus in female somatic cells and, at the same time, provide evidence that both the proband's mother and sister are carriers of the disease.

Both in leukocytes and uncloned fibroblasts the α-galactosidase activity was higher in the proband's mother than in his sister. Furthermore the enzymatic activities in positive clones from the two women differ significantly ($P < .001$). Although both women have in common the X chromosome carrying the mutant α-galactosidase locus, they do have different paternal X chromosomes, so that the difference between the two positive clonal populations could be due to genetic heterogeneity of the two wild-type alleles of paternal origin. We have looked for evidence of structural variation of the enzyme in the positive clones from these two women by studying some functional properties of α-galactosidase. As judged from the fluorometric assay (*11*), the rate of inactivation of the enzyme at 50°C for periods ranging from 1 to 20 minutes was similar for the two positive clonal populations, and the apparent K_m's were not significantly different (3.0 and $2.6 \times 10^{-3} M$). The twofold difference in specific activity, therefore, cannot be ascribed for the moment to genetic heterogeneity.

Using the colorimetric assay described above, we have also determined the activity of α-galactosidase in mixed populations of fibroblasts and epithelioid cells derived from amniotic fluid (*18*) (Table 1). Since α-galactosidase activity is deficient in our proband's fibroblasts, one can reasonably apply this assay to the prenatal diagnosis of Fabry's disease.

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18. Specimens of amniotic fluid obtained between the 14th and 16th weeks of pregnancy were centrifuged, and the pellet was used to establish cell cultures as described for fibroblasts (*3*).
19. G.R. is a fellow of the Leukemia Society of America on leave from the Istituto di Clinica Pediatrica, Università di Bologna, Italy. We thank Mrs. Carol Miller for technical assistance and Drs. Barton Childs, E. C. Heath, and E. Y. Levin for encouragement and advice. Supported by NIH research grant HD 00486.

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Antitumor Activity in Mice of Tentacles of Two Tropical Sea Annelids

Abstract. *Crude extracts of tentacles of two polychaetous annelids completely inhibit growth of Erlich ascites tumor in 60 to 100 percent of treated mice. Dialyzed extracts of one of these annelids,* Lanice conchilega, *show activity in the retentate after pronase digestion, suggesting that antitumor activity is associated with a nonprotein component of the crude tentacle extract.*

During a screening program (*1*) designed to test the pharmacologic activity of ancient Polynesian medicines, tentacles of the tropical sea worm, kaunaoa (*Lanice conchilega*) (*2*), were gathered for antitumor assay at the suggestion of an elderly woman of the Hawaiian race who had experienced many of the Hawaiian medical practices on the island of Molokai (*3*). According to her, cancer patients had shown clinical improvement after drinking an infusion of cooked kaunaoa tentacles daily for several weeks. Which kinds of cancer were treated and the exact results obtained were unknown to the informant. Similar accounts of the anticancer use of kaunaoa body fluid, extracted from the live worm by sucking through a fine bamboo tube, were also found among the Hawaiians in the Kona district of the island of Hawaii. *Lanice conchilega* is shown in Fig. 1, at top.

Worms and tentacles for assay were gathered in 0.5 to 4 m of water in coral reef areas in three sites, two on Oahu, and one at Puako, Hawaii. Whole worms were secured by digging them

out of the sea bottom with a wrecking bar. The retractile tentacles were gathered by quickly tearing them off the living buried worm while the tentacles were extended over the sea bottom for feeding. It was noted that new tentacles grew from the buried worms over a period of weeks, enabling us to "farm" the same worms repeatedly in good collecting areas.

Tentacles and worms were preserved before extraction by immersion in 30 percent ethanol with refrigeration at 5°C. Extraction was done by grinding whole worms or tentacles in a Waring Blendor for 5 to 10 minutes with 55 ml of 30 percent ethanol per gram of solid material used. Some of this volume was the alcohol in which the worms or tentacles were preserved before extraction. After grinding, 82 ml of 30 percent ethanol were added for each gram of original material used. The resulting mixture was shaken intermittently for 24 hours with refrigeration at 5°C except during agitation. The mixture was filtered through fine filter paper and reduced by flash evaporation at 50°C to 0.4 ml per gram of original wet solids. This standardized extract was kept frozen until a few days before use, then in ordinary refrigeration. The *p*H of all the extracts was between 7 and 8; all were neutralized to 7 by the addition of HCl.

For Erlich ascites tumor studies, 141 4-months-old Swiss-Webster strain female mice, each weighing between 25 and 30 g, were used. Tumor was induced by inoculation of 5×10^5 Erlich ascites cells (*4*) intraperitoneally. These cells were freshly drawn with peritoneal fluid from mice inoculated about 7 days previously with Erlich ascites cells. The animals were caged in groups of five. Eighteen to 20 hours after the injection of Erlich ascites cells the mice used for assay were given by intraperitoneal injection maximum nontoxic doses of the test extract.

The maximum nontoxic dose of each extract was determined by injecting varying concentrations of the crude drug into mice not inoculated with tumor cells. The highest concentration at which the animals showed no abnormal behavior or physical findings was considered the maximum nontoxic dose of that extract.

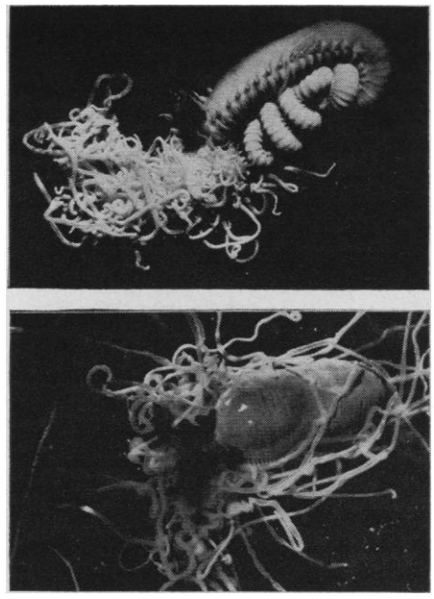

Fig. 1. Kaunaoa (*Lanice conchilega*) (top, three-fourths life size) and *Reteterebella queenslandia* (bottom, nine-tenths life size), showing body, branchiae, and tubular retractile feeding tentacles.

The acute LD_{50} of standardized crude extract of *Lanice conchilega* tentacles given intraperitoneally to mice was 20 ml/kg, or 50 g of tentacles (wet weight) per kilogram. Treatment dosages used for assay were either 1/10 or 1/5 the acute LD_{50}, given twice daily for 2 weeks. At these dosages acute or delayed toxicity did not appear. Doses are noted in volume of extract rather than dry weight; solutions for injection were not dried, to avoid the possible alteration of proteins or their derivatives in drying.

Treatment dosages ranged from 0.5 to 0.10 ml of crude extract; some extracts were given in a dilution of 1 part to 3 parts of water. Mice were under observation each day until they died or were killed. Deaths were recorded daily. Untreated (control) mice all developed ascites, with death following within 21 days. The average time of death was 16 days after injection of Erlich ascites cells.

Granting the possibility of some undetermined "in vitro" effect of our extracts on Erlich ascites tumor development, the intraperitoneal results presented here are offered only as tentative evidence based on a widely used screening procedure.

The results of treatment with *Lanice conchilega* whole worm extract and tentacle extract prepared from specimens from Puako, Hawaii, are shown in Table 1, and are statistically significant; $\chi^2 = 8.57$ and 19.7, respectively, with 1 degree of freedom.

Accurate comparison of effectiveness of whole worm extract and tentacle extract cannot be made from these data because of variation in dosage. However, the protective effect of whole worm extract appears to be roughly similar to that of tentacle extract.

Tentacle extract, prepared as described at the beginning of this report, was also assayed briefly against the splenomegalic effect of Rauscher virus (*5*). Two-tenths of a milliliter of a one-tenth dilution in physiologic saline of Rauscher plasma was inoculated intraperitoneally into ten 6- to 8-week-old male BALB/c mice. Seventy-two hours after inoculation, 0.1 ml of the crude tentacle extract was administered intraperitoneally to five of the animals and repeated on days 3, 4, 5, 6, 7, 10, and 14. On the 21st day the mice were killed, and the spleens were excised and weighed. The ratio of spleen weight in treated mice to control mice was determined. Expressed in percentage, the combined spleen weights of the treated

Table 1. Survival rate of mice injected with Erlich ascites cells intraperitoneally (control groups) and with Erlich ascites cells intraperitoneally plus various sea annelid extracts intraperitoneally (treatment groups) with dosages as indicated.

Group	No. of mice used for each assay	No. developing ascites	No. with no ascites	No. alive at 25 days	Percent alive at 25 days
Control groups	75	75	0	0	0
Treatment groups					
Lanice conchilega, whole worm extract, 0.05 ml twice daily, 2 weeks	10	4	6	6	60
Lanice conchilega tentacle extract, 0.10 ml twice daily, 2 weeks	20	14	6	15	75
Lanice conchilega tentacle extract-fraction A, 0.10 ml twice daily, 2 weeks	5	2	3	3	60
Lanice conchilega tentacle extract-fraction B, 0.10 ml twice daily, 2 weeks	16	12	4	5	31
Reteterebella queenslandia tentacle extract, 0.10 ml once daily for 2 weeks	15	2	13	15	100

animals was found to be 62 percent that of the controls, suggesting that treatment with tentacle extract has a suppressive effect on splenic enlargement at the 21st day. No comparison of survival rates or study of the effect on the late development of leukemia was made, nor are separate spleen weights available.

To determine, if possible, the chemical nature of the active principle of kaunaoa extracts, tentacles were macerated with 30 percent ethanol. The mixture was agitated for 1 day under refrigeration. The cloudy suspension was then filtered and concentrated under reduced pressure at about 50°C. From this, a simple retentate (fraction A) was prepared as follows. Sixty milliliters of crude tentacle solution containing 182.8 mg of solids per milliliter (0.4 ml of solution per gram, wet weight, of tentacles) were dialyzed in a 1-inch (2.54-cm) dialysis bag with constant agitation for 72 hours at 2°C against 2 liters of water changed once. The final retentate volume was 114 ml. Solids were reduced to 8.6 mg/ml or 17.2 mg/ml of the original 60 ml basis.

A "digested" retentate (fraction B) was prepared exactly as was fraction A, with the additional step of subjecting 106 ml of the retentate to the effect of 50 mg of pronase for 1½ hours at 37°C at *p*H 8.1 (1*N* NaOH) and to a temperature of 100°C for 3 minutes, taking 8 minutes to reach 100°C. The resulting solution was reduced at 50°C under vacuum to 53 ml and redialyzed in a 1-inch dialysis tube against 2 liters of water for 45 hours at 2°C with one change of water. Eighty-four milliliters of retentate were suction-filtered with Celite and evaporated at less than 50°C and adjusted to a volume of 53 ml. Dry weight of solids was 7.3 mg/ml.

The results of bioassay of *Lanice conchilega* tentacle fractions A and B are shown in Table 1, and are statistically significant; $\chi^2 = 7.5$ and 7.3, respectively, with 1 degree of freedom.

The survival rates of mice treated with tentacle fractions suggest antitumor activity may be present in a nondialyzable component of crude tentacle extract. The persistence of the antitumor activity after pronase digestion reasonably excludes protein as the active agent.

In August 1969 a similar polychaetous worm with feeding tentacles, *Reteterebella queenslandia*, shown in Fig. 1, at bottom, was collected at Heron Island, on the Great Barrier Reef of Australia, to compare possible antitumor activity with that of *Lanice conchilega*. Preparation of body and tentacle extracts of *Reteterebella queenslandia* was done exactly as with *Lanice conchilega*, providing extracts that were comparable in final volume per wet weight of worm and tentacles.

The body extract of *Reteterebella queenslandia* appears to be somewhat toxic, causing occasional animal deaths during treatment. However, the favorable result of treatment with tentacle extract is shown in Table 1. This is statistically significant; $\chi^2 = 35.0$, with 1 degree of freedom.

Extracts of the tentacles of both *Lanice conchilega*, in Hawaii, and *Reteterebella queenslandia*, in Australia, thus act to protect mice against Erlich ascites cell tumor. Dialysis and enzyme digestion procedures suggest the activity in *Lanice conchilega* tentacles is, or is associated with, either a micelle-forming, or large molecular nonprotein component of the crude extract, based on its retention during dialysis. Although the growth of Erlich ascites cell tumor is fairly sensitive to many foreign materials, the protective effect of these relatively nontoxic sea annelid extracts, considered together with their reputed ancient use in human cancer therapy, is a striking coincidence, certainly worth further study.

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Aspirin: Intestinal Damage in Rats

Abstract. *Lesions induced by aspirin in the small intestine of the rat were visualized after 4 hours by the intravenous administration of a 5 percent solution of pontamine sky blue, 6 BX dye. Dose-response curves in fasted and fed rats indicated that the fed rat was about eight times more susceptible to aspirin-induced intestinal damage than was the fasted rat, while the fasted rat was about 13 times more susceptible to aspirin-induced gastric damage than was the fed rat.*

Production of gastric mucosal damage by aspirin is well documented (*1*), but there have only been a few references to intestinal damage produced by this agent (*2*). In contrast, damage to the small intestine is a well-known side effect for certain other nonsteroid antiinflammatory agents such as indomethacin, phenylbutazone, and flufenamic acid (*3, 4*). A report that a vital aminoazo dye, pontamine sky blue 6 BX (Edward Gurr Ltd., London) was useful in assessing the damaging effect of aspirin on the gastric mucosa (*5*) suggested that this dye might be useful in the detection of intestinal lesions. We report the development of intestinal lesions after oral administration of high doses of aspirin.

Male Holtzman rats (125 to 150 g) were used. "Fasted" animals were deprived of food but allowed free access to water during the 24-hour period before and the 4-hour period after aspirin administration; "free-feeding" animals were permitted free access to both food and water up to the time of death. Aspirin was administered orally as a suspension in 1 percent methylcellulose solution over a wide dose range. Four hours after drug administration, the animals were killed with an intracardiac injection of pentobarbital. Ten minutes before death the animals were injected intravenously in the tail vein with 1 ml of a 5 percent solution of pontamine sky blue 6 BX dissolved in saline. After the animals were killed, the gastrointestinal tract was removed and opened; the gastrointestinal contents were removed by gently wiping with cotton swabs, and the mucosal surface was then examined. The presence of dark blue areas against a pale blue background in both stomach and intestine occurred when the protein-bound dye