pared, and 400 g of 2-benzoylpyridine was added and dissolved. The solution was cooled to 10°C, and 252 g of freshly distilled cyclopentadiene was added dropwise over a 30-minute period. After stirring under nitrogen for 16 hours at 10° to 13°C, the orange-red crystalline product was separated by filtration, washed with cold 1 : 1 alcohol-ether solution, and dried; 322 g (72-percent yield), mp 138° to 160° C; $\lambda_{max}^{CH_3OH}$, 324 mμ (ε, 23,400). The infrared spectrum, combustion analyses (C, H, N, and O), and molecular weight determinations were all consistent with structure V. Fractional recrystallization from ethyl acetate served to separate compound V into geometric isomers which melted at 175°–76°C and 181°–82°C, respectively.

Fulvene V reacts readily with maleimide to give 5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (McN-1025, VI). Thus 5.4 g of V (mixed isomers) and 1.26 g of maleimide were dissolved in 25 ml of benzene and heated under reflux for 4½ hours. After cooling in an ice bath, filtration, concentration of the filtrate, cooling, and filtration, a stable, white crystalline solid, mp 190°–98°C; $\lambda_{max}^{CH_3OH}$, 250 mμ (ε, 17,500), identified as VI (5.9 g, 90-percent yield), was obtained. Combustion analyses (C, H, and N), the infrared spectrum, and nuclear magnetic resonance spectrum give support to the structure designated.

Adduct IV and several analogous compounds (*4*) were much less toxic to rats. Results obtained so far have shown that relatively minor changes in the structure of VI give compounds which are much less toxic to rats. For example, the mixture of the four possible stereoisomers of compound VI (two *endo* and two *exo*isomers) have been separated. Two of the isomers (60 to 70 percent of the total mixture) appear to be far more toxic to rats than the other two.

The carboximide is a substance which is selectively toxic to the genus *Rattus*. It does not produce significant changes in species other than the rat, even at extremely high doses. This high specificity indicates that even closely related species manage this chemical in different ways and suggests an underlying physiological difference between rats and other species.

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Growth-Inhibiting Agents from Mercenaria Extracts: Chemical and Biological Properties

Abstract. *Preliminary studies of the chemical nature of an antitumor agent from the common quahog,* Mercenaria mercenaria, *are reported. The agent has a regressive and an inhibiting effect on sarcoma 180 and on Krebs 2 carcinoma in Swiss albino mice.*

The effect of constitutents of normal cells on malignant growths has recently received renewed interest in the work presented by Szent-Györgyi *et al.* (*1*). These workers have suggested that the term "autobiotics" be used for such tumor-inhibiting substances (*2*). The fact that cellular extracts of many origins have shown both a stimulating and inhibiting effect on cancer cells has been studied for a number of years in these laboratories (*3*), while the action of normal tissue extracts from spleen, brain, and liver has been reported from the Institutum Divi Thomae Research Laboratories over the past 20 years (*4*). Preliminary studies have been made of extracts from several different kinds of marine invertebrates as possible sources of antitumor activity (*5*). The most promising extracts, as demonstrated by their effect on sarcoma 180 in female Swiss albino mice, were those obtained from the common edible quahog, *Mercenaria mercenaria.* In this report I present some of the results of an investigation of the chemical and biological nature of the active principle believed to be responsible for the antitumor activity of the extracts.

The active *Mercenaria* agent, which can be extracted with water (*6*), is not precipitated by 20 to 25 percent saturation with $(NH_4)_2(SO_4)$, but greater concentrations of this salt decrease the yield of active agent in the supernatant. Seventy percent of activity can be extracted in the supernatant when the extract is treated with four volumes of methyl alcohol chilled to −20°C and the extract chilled to +2°C. At room temperature, 15 to 20 percent of the activity can be found in the supernatant. The agent is destroyed by boiling at 100°C for 25 minutes, its activity is decreased by heating at various temperatures above 50°C for 25 minutes, but 100 percent of the activity is retained in extracts heated to 37°C. The substance is nondialyzable, and lyophilyzation at −20°C does not destroy the antitumor activity. Partial purification of the active material has been achieved by chromatography on Sephadex-gel columns. Lipids do not appear to be responsible for the antitumor activity, as shown by testing the cellulose acetate strips obtained after electrophoresis with Nigrosine. Extracts of *Mercenaria* in previous experiments inhibited the growth of sarcoma 180 and had no toxic effects in the dilutions used. While untreated mice (controls) died within 10 days after implantation of tumor—by the trocar method in the axillary region—animals treated with extract were still healthy and normal after 6 months. These animals, which were kept so that the long-range effects of the extract could be determined, showed no evidence of the recurrence of tumors and produced normal litters (*6*).

Mercenaria extracts also inhibited the Krebs-2 ascites tumor in female Swiss mice, 3 to 4 weeks old. Fifteen control animals and groups of 10 experimental mice were each injected with 0.25 ml of ascites fluid in the right axillary region. Four days later, animals without tumors were rejected, and the remaining mice in the experimental groups were injected subcutaneously in the left axillary region, once daily for 7 days, with *Mercenaria* extract partially purified on Sephadex-gel columns. One unit of extract (*7*) was injected per day in a total volume of 0.25 ml. Control mice received 0.25 ml of normal saline daily. Extracts in concentrations higher than those used were toxic. This toxicity, which was probably due to the potassium present, could be

Table 1. Partial purification of antitumor agent in *Mercenaria* extracts on Sephadex-gel columns. Results obtained with Swiss mice implanted with Krebs 2 carcinoma.

Sephadex gel column	Antitumor activity in vivo	Units of activity injected in sample	Mean wt. of tumor (mg)
	Treated		
G-100	20	63	1742±125
G-75	25	63	1413±480
G-50	30	63	1382±83
G-25	67	43	1340±400
	Controls		
G-75, G-50, and G-25			2096±715
G-100			2088±150

eliminated by dialysis for 24 to 36 hours against distilled water at 4°C.

Preparation and partial purification of the crude extract was as follows. Fresh *Mercenaria* were removed from the valves and homogenized in a Waring blender after the addition of distilled water and precipitation with 20 percent ammonium sulfate solution. The homogenate was centrifuged at room temperature at 5000 rev/min for 15 minutes. The supernatant was then dialyzed for 24 to 36 hours against distilled water at 4°C. The crude material was concentrated to a powder by freeze-drying at −20°C and the powder was stored at −10°C until used.

For further, but incomplete, purification, 5-ml samples containing a predetermined number of mouse units of extract were reconstituted and introduced onto a Sephadex-gel column with a void volume of 45 ml. An appropriate buffer of neutral 0.1*M* NaCl was used as the elution fluid. Approximately 125 to 150 ml of the fractionated sample were collected at room temperature over a period of 2 to 3 hours. The total collection was lyophilyzed at −20°C, and then reconstituted with 0.1*M* NaCl and used to treat test animals for 7 days.

Of the Sephadex gels tried (G-100, G-75, G-50, and G-25), the fractions from the G-25 produced the greatest amount of active antitumor substance per milliliter of extract sample and per number of units introduced onto the column. This would seem to suggest fractionation in the molecular-weight range of less than 10,000 grams.

Partial purification to date seems to indicate almost pure inhibitor. There seems to be no great traces of promotor present to suppress the activitity of the inhibitor material. Further purification may indicate the presence of a growth promoter (*1, 2, 8*). Fortunately many of the difficulties encountered with mammalian tissues, in which the action of inhibitors is compensated by promotors, have been eliminated.

These studies seem to indicate a new agent widely distributed in nature, especially in marine fauna. During the summer months this agent can be found in concentrations eight to nine times as great as that extracted from *Mercenaria* during the remainder of the year. Antitumor activity may be due to some metabolic agent or agents present in *Mercenaria* that is active as an antitumor compound or compounds. These substances may be useful as therapeutic and prophylactic agents in the treatment of cancer.

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Intestinal Phosphatase Activity: Acceleration of Increase by Puromycin and Actinomycin

Abstract. *Alkaline phosphatase activity of the mouse duodenum normally increases 20-fold between 13 and 20 days. Activity at 15 days is raised two to three times above normal by administering actinomycin D, puromycin, or the aminonucleoside of puromycin at 13 or 14 days. Phosphatase activity in the jejunum and kidney are not similarly affected.*

In the duodenum of the young mouse, the specific activity of alkaline phosphatase, measured in whole homogenates, increases approximately 20-fold during the 3rd week of postnatal life (*1*). This increase, which is dependent on the secretion of adrenocorticoids (*2*), is accompanied by changes in the characteristics of the enzyme activity. One of the most striking changes is an increase in the rate at which phenyl phosphate (PhP) is hydrolyzed in comparison with the rate at which β-glycerophosphate (bGP) is hydrolyzed; the ratio of rates of activity on the two substrates (PhP/bGP) rises from less than 1.0 near the end of the 2nd week to more than 3.0 at the end of the 3rd week (*3*). These events are not due to loss of dissociable inhibitors or to gain of dissociable activators (*4*).

This study was undertaken to investigate the possibility that the increase of activity is due to rapid synthesis of new enzyme molecules. The antibiotics puromycin dihydrochloride and actinomycin D are known to interfere with protein synthesis, puromycin by interrupting the formation of peptide chains (*5*), and actinomycin D by inhibiting the DNA-dependent synthesis of RNA (*6*). Both puromycin (*7, 8*) and actinomycin D (*8, 9*) prevent increases of certain enzyme activities in mature tissues, and puromycin has been shown to have the same effect during developmental stages (*8, 10*). The fact that the aminonucleoside of puromycin may interfere with enzyme-dependent processes without having a parallel effect on protein synthesis (*11*) led to the use of this compound as well (*12*).

Litters of nine young mice were used: three were killed at 13 or 14 days; three were injected subcutaneously with actinomycin D or aminonucleoside dissolved in saline or with puromycin dihydrochloride in 1.39 percent bicarbonate buffer at *p*H 7.3 (*11*);