

32 have been detected in oyster samples from Willapa Bay. Plans are under way to separate chemically and to measure the concentration of phosphorus-32, scandium-46, chromium-51, manganese-54, iron-59, cobalt-60, strontium-90, and some of the long-lived rare earths (which are present in Columbia River water) in West Coast sea foods (12).

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Calvacin: A New Antitumor Agent

The development of a new oncostatic agent, recently named "calvacin," arose from the earlier findings of Lucas *et al.* (1). They reported the presence of unknown oncostatic principles in aqueous extracts of sporophores of giant puffball mushrooms, species *Calvatia gigantea*. They also observed that shake-flask cultivation of certain mycelial strains, derived from *Calvatia* sporophore tissue, led to the elaboration of an antitumor substance (or substances) in the medium.

These preliminary observations formed the basis of a more extensive program to isolate the active agent (or agents) present in the mushroom and to develop a submerged fermentation process which would lead to the development of a more convenient and reproducible source of calvacin. Active participation

in the program was undertaken by the central research department of Armour and Company, the departments of horticulture and chemistry, Michigan State University, and the division of experimental chemotherapy, Sloan-Kettering Institute for Cancer Research. Isolation of calvacin from sporophores and the development of the fermentation process were the responsibility of the Armour groups. The development of new *Calvatia* strains which could provide higher yields of calvacin, and other related nutritional studies, were undertaken at Michigan State University. All test samples generated from these research efforts were submitted to the Sloan-Kettering group, who determined their antitumor activity in the standard sarcoma-180 mouse assay (2).

Results of more than 2 years of research have led to the isolation from sporophores, by two independent techniques, of a highly purified mucoprotein fraction with a specific S-180 activity of 300 to 500 calvacin units per milligram (3). The screening of several hundred mycelial cultures led to the isolation of *Calvatia gigantea* strains which provided both higher yields of

Table 1. Tumor spectrum studies, in mice, rats, and hamsters, on calvacin derived from *Calvatia gigantea*. (—) No effect (tumors in treated animals developed to three-fourths, or more, the size of those in controls); (=) slight inhibition (tumors in treated animals developed from one-fourth to three-fourths the size of those in controls); (+) marked inhibition (tumors in treated animals developed to one-fourth, or less, the size of those in controls).

Tumor	Effect of calvacin (1 mg/kg/day)
Sarcoma-180	±
Sarcoma-180 (ascitic)	+
Sarcoma MA 387	+
Mammary adenocarcinoma E 0771	±
Mecca lymphosarcoma	±
Ridgeway osteogenic sarcoma	±
Bashford carcinoma 63	—
Carcinoma 1025	—
Ehrlich carcinoma	±
Ehrlich carcinoma (ascitic)	—
Lewis bladder carcinoma	±
Lewis lung carcinoma	±
Glioma 26	—
Friend virus leukemia	—
Friend virus leukemia (solid form)	±
Harding-Passey melanoma	±
Leukemia L 4946	—
Jensen rat sarcoma	—
Murphy-Sturm lymphosarcoma	—
Walker carcinosarcoma 256	±
Flexner-Jobling carcinoma	Toxic
Crabb hamster sarcoma	+
Fortner adenocarcinoma (small bowel)	—
Fortner adenocarcinoma (pancreas)	—

calvacin and shorter fermentation periods. In addition, both laboratory (14 lit.) and pilot-plant (230 to 1200 gal) fermentation processes have been developed. Scale-up of the fermentation process to the pilot-plant stage and the subsequent recovery of calvacin from beer (on cellulose ion-exchange columns) were carried out, with results comparable to those of the laboratory process.

A purified fraction derived from sporophores of *Calvatia gigantea* (200 calvacin units per milligram) was subjected to a broad-screen tumor survey (see Table 1) and was found to possess antitumor activity against 14 of 24 various mouse, rat, and hamster tumors.

Physicochemical studies of calvacin derived from either sporophores or fermentations indicate that the products are identical or essentially similar in nature. Present knowledge of the chemical nature of calvacin indicates that it is a nondiffusible, basic mucoprotein. Calvacin is moderately heat-stable and is amenable to organic solvent precipitation or treatment with anionic and cationic cellulose ion-exchange materials. (4)

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3. One calvacin unit per milligram is defined as the potency of a *Calvatia* fraction that causes a 25-percent inhibition of mouse sarcoma-180 (by measurement of tumor diameter) when tested at a dosage level of 1.0 mg per mouse per day under standard conditions. The purified fractions show significant activity at 2.0 to 3.3 µg per mouse per day.
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