As noted above, seromucoid from normal rabbit serum produced no Cx-protein response; that from serum obtained 24 to 48 hours after injection of adjuvant was uniformly active in this regard; and that from serum obtained 24 to 48 hours after 500 r whole-body x-irradiation was also active. Seromucoid obtained earlier than 24 hours after irradiation was irregularly active. Activity was commonly but not invariably associated with sera which gave the highest yields of seromucoid.

Other physiological activities of Co-Cx-protein have also been noted. Besides eliciting a Cx-protein response on intravenous injection, Co-Cx-protein elicited an anamnestic type of response on injection into the immunized rabbit. For example, in Fig. 1 is shown the Cx-protein response and the immune response of a rabbit given 1 mg of tobacco mosaic virus (*6*) intravenously, without adjuvant, followed on the 42nd day by 2.5 mg of Co-Cx-protein prepared from post adjuvant serum. Similar anamnestic responses to Co-Cx-protein have been obtained in rabbits immunized to BSA and employing Co-Cx-protein isolated electrophoretically, as well as to seromucoid prepared by Winzler's method. The absolute numbers of circulating lymphocytes were invariably decreased 50 to 75 percent in 4 to 6 hours following intravenous injection of seromucoid fractions which elicited a Cx-protein response. Very frequently a compensatory increase in heterophils also occurred to give an over-all leucocytosis. Another property of Co-Cx-protein which has been noted is its toxicity in the rabbit after blockade of the reticuloendothelial system with colloidal thorium dioxide (Thorotrast) (*7*). Injection of Co-Cx-protein 2 to 3 hours after intravenous injection of a nonlethal dose of Thorotrast was invariably fatal within 24 hours. Normal seromucoid did not enhance Thorotrast toxicity.

The activities associated with acute phase seromucoid could be ascribed to a new humoral factor, or to the appearance in this fraction of toxic tissue polysaccharides, or endotoxin. While we incline toward the latter view, more extensive comparisons are being made to establish this with certainty. With the possibility that endotoxin is released shortly after whole body irradiation and because of the marked enhancement of the toxicity of injected endotoxin administered during reversible hemorrhagic shock (*8*), it will be desirable to assess the toxicity of postirradiation serum in reversible hemorrhagic shock (*9*).

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Zinc-65 and Chromium-51 in Foods and People

Abstract. Some radioisotopes introduced into the Columbia River via effluent water from reactors at Hanford, Wash., are found in crops irrigated with this water and in sea food harvested near the mouth of the river. Measurements of zinc-65 and chromium-51 in foods and in individuals consuming these foods are reported and are compared with zinc-65 concentrations resulting from fallout.

The use of water as a coolant for reactors at Hanford, Wash., with its subsequent disposal to the Columbia River, results in the introduction of several radioisotopes into the river. The half-lives of some of these are sufficiently long to make it possible to trace their distribution from the river through the produce of irrigated farms to man, and through the food chains of the aquatic life of the river (*1*). A report describing the distribution of zinc-65 in some produce from farms irrigated with Columbia River water, and in individuals utilizing these materials, was published earlier (*2*). Recently some Zn^{65} concentrations present in foods as a result of radioactive fallout were reported by workers in other laboratories (*3*). During 1959 and early 1960, a more comprehensive study of the Zn^{65} concentrations in produce from farms irrigated by the Columbia River and in locally available commercial sea foods was made. These concentrations have been compared with those found in foods from other locations in the world.

A NaI(Tl) scintillation well crystal detector 9⅜ inches in diameter (*4*) (which would hold a 500-ml polyethylene sample bottle) and a multichannel analyzer were used for the measurements, unless otherwise stated. The Cr^{51} concentrations were also determined, where possible, by direct gamma-ray spectrometric analysis. The samples of produce from farms irrigated with Columbia River water were obtained from an irrigation project about 30 miles downstream from the Hanford reactors. Samples of produce from two farms, one of which used sprinkler irrigation and the other ditch irrigation, were collected during the summer of 1959. The Zn^{65} and Cr^{51} concentrations observed in produce from these farms and in produce from other areas are tabulated in Table 1. Although it was not possible to obtain the same crops from the two farms for comparison, it is apparent that levels of Zn^{65} and Cr^{51} are generally higher in the crops from the sprinkler-irrigated farm, and that levels in the samples of tomatoes, carrots, green beans, corn, and alfalfa, which were obtained from both locations, were higher in samples from the sprinkler-irrigated project. This would indicate that direct foliate absorption through the leaves of sprinkler-irrigated crops is playing an important role in the uptake of Zn^{65} and Cr^{51}.

Studies at the Washington State Irrigation Experiment Station at Prosser have shown that soils in this area are deficient in available zinc (*5*). This condition could enhance the uptake of Zn^{65} by either method of irrigation.

The pasture grass listed with the produce from the ditch-irrigated farm (Table 1) has a much higher Zn^{65} content than grass from the sprinkler-irrigated farm, but this finding is undoubtedly related to the amount of irrigation, for this land was irrigated almost continuously. Milk samples were not available from this farm during the summer of 1959; however, those obtained during 1958 (*2*) showed about two to three times the Zn^{65} listed for the sprinkler-irrigated farm. The Cr^{51} concentrations listed in Table 1 are comparable with the levels of Zn^{65} in several of the crops, but Cr^{51} was not observed in milk and meat. The absence of Cr^{51} in milk and meat, plus its relatively short half-life, limits its availability to human beings. To date, Cr^{51} has not been positively identified in individuals from this area by total-body counting.

The individual who operated the sprinkler-irrigated farm and whose diet consisted mainly of the foods included in Table 1 was counted on 30 August 1959 in the Hanford whole-body counter [similar to that developed by Marinelli *et al.* (*6*)]. His Zn^{65} content was about 0.2 μc, and no Cr^{51} was observed. For comparison, 6 μc is the exposure limit recommended by the National Committee on Radiation Protection (*7*) for persons in the neighborhood of a controlled area.

The Zn^{65} concentration in milk from the sprinkler-irrigated farm was meas-

ured periodically from 16 July 1959 through 28 October 1959 and was found to average 1.9 $\mu\mu$c/ml. A family of six, including four children from 14 months to 8 years of age, which had consumed 1 gal of milk a day (average 0.0073 μc/gal, varying from 0.0045 to 0.0099 μc/gal) from this source for approximately 7 months, were counted in the total-body monitor on 12 October 1959. The milk was the only known source of Zn^{65} in their diet. The whole-body counter showed the average Zn^{65} content of these six individuals to be 0.047 μc, varying from 0.037 to 0.066 μc per person. The Zn^{65} content appeared to be related to the amount of milk consumed rather than to the age of the individual. Calculations, based on an effective half-life for Zn^{65} of 125 days for man (*8*) in the secular equilibrium equation, show that 31 percent of each day's intake of Zn^{65} must be retained in the body. This value may be somewhat low, since the fodder for the cows contained a smaller fraction of pasture grass during the spring months.

Table 1. Concentrations of zinc-65 and chromium-51 in farm produce (in $\mu\mu$c/g).

Sample	Zn^{65}	Cr^{51}
Sprinkler-irrigated farm (Columbia River water)		
Alfalfa	8.9	
Pasture grass	3.9	6.9
Soil (to depth of 2 in.)	5.6	38.4
Milk	1.9	<0.23
Corn	2.1	
Green beans	1.3	
Okra	0.59	
Carrots	0.36	1.0
Lettuce	0.45	1.2
Tomatoes	0.36	0.81
Boysenberries	0.29	0.67
Cantaloupe	0.28	
Cucumbers	0.28	
Watermelon	0.22	
Eggs	0.22	
Grapes	0.19	0.86
Cabbage	0.19	0.77
Zucchini squash	0.15	
Hamburger*	4.6	<0.23
Pork, flesh*	0.35	
Pork, fat*	0.27	
Pork, bone*	0.90	
Ditch-irrigated farm (Columbia River water)		
Pasture grass	36.0†	8.0
Wheat	4.0	0.81
Mint	2.8	
Corn	1.6	
Alfalfa	0.90	
Cabbage	0.28	
Carrots	0.17	0.29
Beets	0.16	0.14
Potatoes	0.14	
Green beans	0.095	
Strawberries	0.095	
Raspberries	0.090	
Onion tops	0.077	
Red beet tops	0.077	
Sugar beets	0.063	
Tomatoes	0.035	
Irrigation water	0.072	3.2
Other Locations		
Corn	0.016	Calif.
Meat (beef)	0.0090	Yakima, Wash.
Tomatoes	0.0072	Calif.
Green beans	0.0036	Calif.
Carrots	<0.002	Spokane, Wash.
Lettuce	<0.001	Calif.

* Calculated for the date the sample was received. † This pasture grass was irrigated almost continuously.

Zinc-65 is known to be a constituent of fallout, and it has been shown that Zn^{65} from fallout is present in people (*9*). To compare the levels of fallout-produced Zn^{65} in foods from other areas and in the produce from the Columbia River irrigation projects, foods from other localities were analyzed. The concentrations, listed at the bottom of Table 1, are lower by two to three orders of magnitude than those in samples from the local irrigation projects. The concentrations were measured by chemically separating the zinc from approximately 3-kg samples and counting it in a well crystal 5 in. in diameter and 5 in. high. If the Zn^{65} content of these foods is representative of foods in the diet of people of the western United States, it would be expected that this population would obtain less Zn^{65}, by two to three orders of magnitude, from farm produce than the individual whose diet consists of foods from the sprinkler-irrigated farm. Actual measurements with the whole-body counter of Zn^{65} in about 200 individuals, most of whom worked at the Hanford projects but were not exposed to Zn^{65} in any known way other than from fallout, showed body contents averaging about 0.0036 μc.

In the measurement of radioisotopes in people at Hanford it was found that the Zn^{65} in at least one individual could not be traced to farm produce from the Columbia River irrigation projects and was too high to be attributed to fallout. An examination of possible sources of this radioisotope revealed that oysters, which were a frequent part of the individual's diet, contained Zn^{65}.

In view of this finding, a program was set up to measure the Zn^{65} in sea foods from the local markets. About 50 samples of sea foods, including oysters, crabs, tuna, salmon, clams, shrimp, sardines, anchovies, codfish, herring, and halibut, were measured by counting them directly in the 9¾-in. well crystal (see Table 2). The sample sizes varied from about 100 to 500 g, and the corresponding detection limit for the large crystal varied from about 0.05 to 0.25 $\mu\mu$c/g for 30-minute measurements. Of the 14 oyster samples measured, nine were found to contain measurable amounts of Zn^{65}. Except for samples 2 and 3, only the packing or distributor location is known, but all of the samples measured containing more than 0.5 $\mu\mu$c/g originated on the West Coast of the United States.

Table 2. Zinc-65 in oysters from local markets.

Sample No.	Packing	Origin	Zn^{65} ($\mu\mu$c/g)
1	Fresh	West Coast	63.5
2*	Fresh	Willapa Bay, Wash.	56.3
3†	Fresh	Willapa Bay, Wash.	37.8
4	Fresh	South Bend, Wash.	38.1
5	Canned	West Coast	15.1
6	Canned‡	Seattle, Wash	4.13
7	Canned	Gulf of Mexico	<0.1
8	Canned	New Orleans, La.	0.30
9	Canned	Biloxi, Miss.	<0.1
10	Fresh	Port Norris, N.J.	0.19
11	Canned	Japan	0.19
12	Canned	Japan	<0.2
13	Canned	Japan	<0.1
14	Canned	Japan	<0.2

* Sampled 5 Sept. 1959 from Willapa Bay. † Sampled 27 Jan. 1960 from Willapa Bay. ‡ Oyster stew.

Samples 2 and 3 were obtained directly from the oyster beds in Willapa Bay near Bay Center, Wash. Approximately half of the oysters produced on the West Coast are grown at Willapa Bay (*10*), which is located about 30 miles north of the mouth of the Columbia River. A sea-water sample collected over the Willapa Bay oyster beds contained 0.0002 $\mu\mu$c of Zn^{65} per milliliter. If this single sample is representative, the oysters concentrate the Zn^{65} by a factor of about 2×10^5, the same value reported by Chipman *et al.* (*11*) for nonradioactive zinc.

Although the Zn^{65} concentration in oysters from Willapa Bay is relatively high compared with that from other locations, the added exposure which an individual would receive from eating 1 pint of such oysters every week would amount to less than 10 percent of the exposure from background radiation which an individual receives continuously from natural sources. [By using the Handbook No. 69 parameters (*7*), it is estimated that a continuous intake rate of 2.2 μc/day would result in a dose of 5 rem/yr to the body—assuming that the natural background is 100 mrem/yr].

The Zn^{65} concentrations observed in the other sea foods were much lower than those found in the oyster. Although Zn^{65} was detectable in about half of these samples, the concentration in all but two was below 0.5 $\mu\mu$c/g. These two were a tuna sample (1.1 $\mu\mu$c/g) processed in Astoria, Ore., and a clam sample (2.8 $\mu\mu$c/g) obtained from Willapa Bay.

This study is being continued, with larger samples where necessary, to obtain a reasonably accurate measurement of these trace quantities of Zn^{65}. Although Zn^{65} is by far the major gamma-ray-emitting radioisotope observed in sea foods, other radioisotopes are known to be present. Cobalt-60 and phosphorus-

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 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*).

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)	−

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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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