we found increased amounts of the normal fraction A_2. Because of the interesting relations of this fraction with hemoglobin E and thalassemia (*10*), plans are being made for the study of this fraction in a quantitative and systematic way in future surveys (*11*).

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26 July 1960

Appearance of a Substance in Acute Phase Serum Which Elicits Cx-Protein Responses

Abstract. Seromucoid was isolated from serum of rabbits during the acute inflammatory period following subcutaneous injection of an irritant and at times after whole body irradiation. It produced a Cx-protein response on intravenous injection into normal rabbits, an anamnestic response in immunized rabbits, leucocyte changes characteristic of inflammation, and marked toxicity in rabbits with reticuloendothelial system blockade. Normal seromucoid did not possess these properties.

C-Protein (CP) and Cx-protein (CxP) are abnormal serum proteins which appear in serum of man and rabbit, respectively, in response to various noxious stimuli. They appear within 24 hours of application of a sufficient stress and disappear as rapidly with its termination. Their function is unknown, although C-protein has been reported to increase leucocyte mobility, and rabbits which produce high titered antibody give stronger Cx-protein responses than those producing lower titered antibody (*1*). Cx-protein apparently enhances the inflammatory tissue response to noxious agents (*2*). Their source and mechanism of appearance in serum is unknown although the reticuloendothelial system has been implicated (*3*).

A second abnormal substance(s) has been found in serum which appears coincidentally with Cx-protein 24 hours after subcutaneous injection of mineral oil-Aquaphor emulsion (incomplete adjuvant) and at various times after 500 r of whole body irradiation (*4*). We refer to it as Co-Cx-protein. It was first recognized by its ability to elicit a Cx-protein response on intravenous injection of small amounts into normal rabbits. Such activity was not associated with comparable fractions of normal serum.

Material with this activity is prepared by Winzler's method for the isolation of seromucoid (*5*) from the fraction of proteins which precipitate from acute phase serum with ammonium sulfate between one-half and three-fourths saturation. Table 1 summarizes the properties of a number of such preparations.

Table 1. Properties of various seromucoid preparations.

Source rabbits	Samples (No.)	Cx-protein titer* of serum source	Yield of seromucoid (mg/100 ml)	Dose injected in test rabbits† (mg)	Cx-protein titer of test rabbits 24 hr after injection
Normal	6	neg.–trace	3.5–6.7	0.35–2.1	5 neg., and 1:2
24–48 hr post adjuvant	4	1:8–1:16	13–21	1.2 –5.0	1:4–1:64
1 hr post irradiation	2	neg.	6.6–9.4	1.3 –1.9	1:4, 1:8
3 hr post irradiation	3	neg.–trace	5.4–9.7	1.1 –2.0	neg., 1:2, 1:2
6 hr post irradiation	3	neg.–1:8	5.8–16	1.2 –3.2	neg., neg., 1:2
12 hr post irradiation	2	1:2–1:8	13–19	2.5 –3.8	neg., 1:1
24–48 hr post irradiation	4	1:4–1:16	13–25	1.1 –5.1	1:1–1:8

* Cx-protein titers are recorded as the highest dilution of serum giving a positive test with specific antiserum in capillary precipitin tubes (*10*). † Usually the dose selected was equivalent to 20 ml of donor serum, although active samples were tested in lower doses and inactive samples at higher doses on repetitive tests.

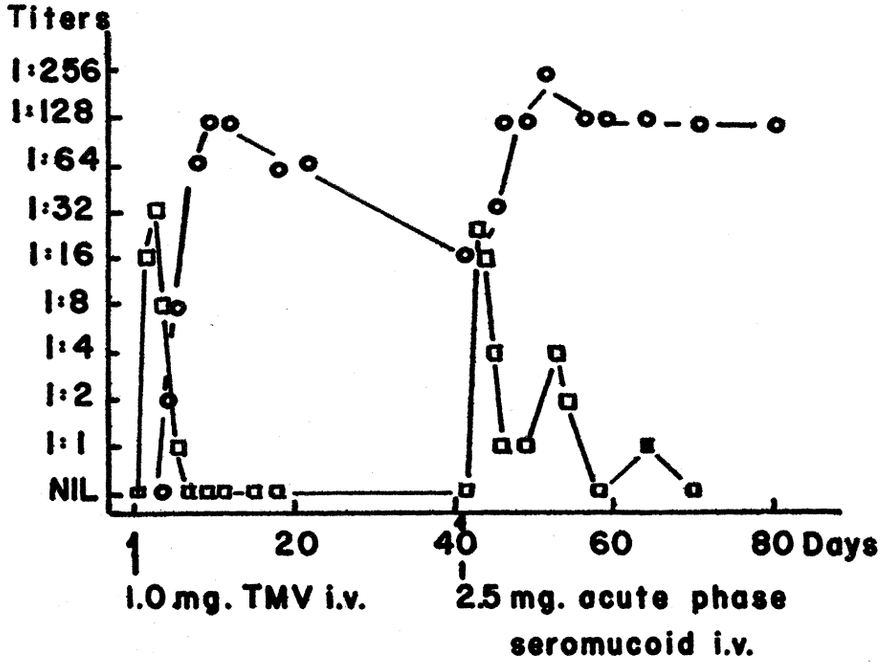

Fig. 1. Cx-protein and anti-tobacco mosaic virus titers following injection of 1.0 mg of tobacco mosaic virus (first arrow) and 2.5 mg of seromucoid (second arrow). The Cx-protein levels are denoted by squares, and anti-tobacco mosaic virus levels by circles.

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-