Reports

Proteolytic Enzyme Activity in Irradiation-Sterilized Meat

The recorded formation of tyrosine crystals in the storage of irradiation-sterilized raw meat (*1*) is indicative that a general proteolysis occurs in irradiated meat samples (tyrosine is the least soluble of the amino acids). The principal proteolytic enzymes present in beef muscle have been identified as cathepsins (*2*). It has been reported that an irradiation dose of 1.6 million rep inactivates only 50 percent of the proteinase activity in samples of beef muscle (*3*).

Additional pertinent information is provided by data taken from three different investigations in the Radiation Preservation of Foods Project at the Quartermaster Food and Container Institute for the Armed Forces (*4*).

Extensive crystal formation gave a very unappetizing appearance to all samples of irradiated pork tenderloin that had been stored for 3 months at 100°F. Samples stored at 72°F had an increased free amino acid content in the fluids that were squeezed from the meat, but enzyme activity had not produced sufficient concentration of tyrosine to form crystals. Variables in the study were an initial freezing or wet-ice pack before irradiation, an irradiation dose of 2 or 3 million rep, and storage at either 72° or 100°F.

Table 1 shows the results obtained from paper-chromatographic, amino-acid analyses of residue fluids after steam-distillation of various samples of ground beef. The preirradiation heat treatment was based on conditions determined to be sufficient for inactivating catalase by heat and consisted of heating the meat in a steam retort and holding it for 10 minutes at an internal temperature of 160°F. The results show that proteolysis has been inhibited in the meat in which the enzymes were heat-inactivated.

One milliliter of 1-percent solutions of ascorbic acid or of cysteine (known cathepsin activators) and 1 ml of copper sulfate or of hydroquinone (cathepsin inhibitors) were added to 200-g samples of ground beef prior to irradiation at 3, 6, or 12 million rep. Some samples were stored at 76°F, and the rest at 100°F.

No tyrosine crystal formation was evident in sample cans that were opened after 3 months' storage. After 7 months' storage at 100°F, however, crystals were found in the 3-million-rep-dose cans containing the added cathepsin activators. No crystals were observed in the cans used to test the other variables. These results may be interpreted as follows:

1) The rate of enzyme activity is accelerated at the higher storage temperatures.

2) The inhibition of enzyme activity at greater doses of irradiation may be due to destruction of the enzyme activators. More likely, however, this work confirms the report (*3*) that proteinases exhibit greater resistance than bacteria to inactivation or destruction by irradiation. The effect is opposite to that encountered in heat sterilization of foods where the amount of heat necessary to inactivate enzymes is less than that required to destroy microorganisms.

3) A supplementary confirmation is made of cathepsins as the principal proteolytic enzymes present in beef muscle.

The data cited show that prolonged storage and storage at elevated temperatures will destroy meat structure and probably develop a bitter taste in it (most L-amino acids are bitter). The necessity for inactivation of the proteolytic enzymes is indicated, therefore, if irradiation-sterilized meat is to become an acceptable food product.

Table 1. Semiquantitative paper-chromatographic, amino-acid analyses of residue fluids from steam distillations of equal weights of fresh and irradiated samples of ground beef. The presence of free amino acids is indicated by +.

Treatment	Presence of free amino acids	
	Before storage	After storage
Fresh	+	
Irradiated (2×10^6 rep)	+	++++*
Irradiated (3×10^6 rep)	+	++++*
Preirradiation heat-treated (3×10^6 rep)	+	+†

* Stored 3 months at 76°F; † stored 5 months at 76°F.

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References and Notes

1. G. B. Pratt and O. F. Ecklund, *Food Technol.* 10, No. 10, 496 (1956).
2. A. K. Balls, *Ice and Cold Storage* 41, No. 4, 85 (1938).
3. D. M. Doty and J. P. Wachter, *J. Agr. and Food Chem.* 3, 61 (1955).
4. This report is paper No. 681 in a series of papers approved for publication. The views or conclusions are ours and are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense. A fuller account of the several studies on which this report is based is in preparation.

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Effect of Ionizing Radiation on Rust Reaction in Plants

Studies on the nature of resistance of plants to rust diseases have long been hampered by the fact that the rust fungi are obligate parasites. One approach to the problem is the alteration of the disease reaction by manipulation of environment (*1*) or by chemical treatment (*2*) of the host plant, followed by appropriate physiological or biochemical analyses of the change induced. Recently, ionizing radiation (*3*) has been investigated as a possible therapeutic agent and as a tool for studying the vascular wilt disease of tomato, which is caused by the heterotrophic fungus, *Fusarium oxysporum* f. *lycopersici.* This report (*4*) records the effects of chronic gamma- and acute x-ray treatments on the host-parasite interaction in several rust diseases: flax rust (*Melampsora lini,* race 1); wheat stem rust (*Puccinia graminis tritici,* races 15B and 111); oat stem rust (*P. g. avenae,* race 7A) and crown rust of oats (*P. coronata avenae,* race 202).

For chronic gamma treatments, seedlings grown to the first- or second-leaf stage in plastic pots were exposed to radiation from a 9.4-c Co^{60} source in the greenhouse. Dose, dose rate, stage of growth, and time of inoculation were the main variables. For x-ray treatments, the seedlings were grown in a mixture of loose soil and peat, removed, washed and enclosed in plastic film for irradiation. Plants were inoculated immediately after irradiation and transplanted to soil or grown in liquid nutriculture. Lead shielding and x-rays were used for partial-plant exposures.

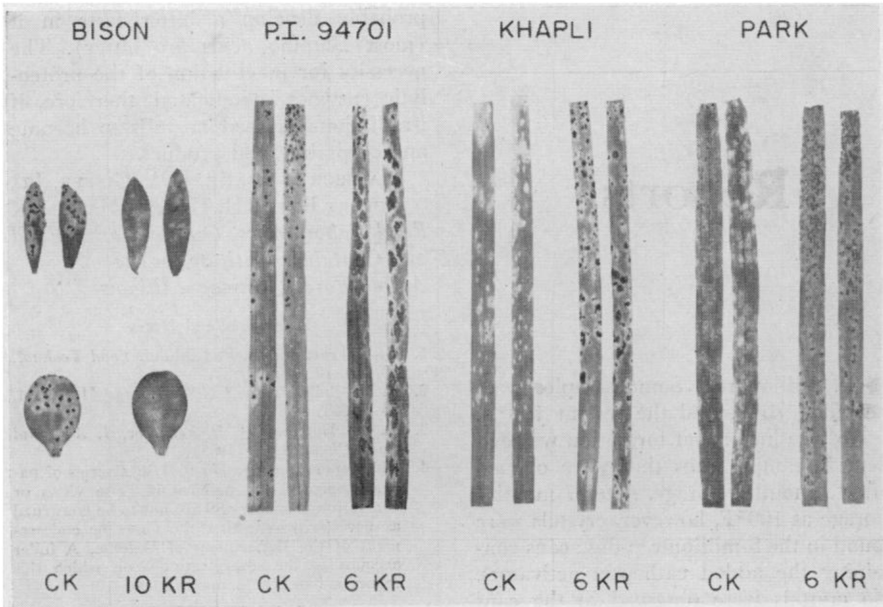

Fig. 1. Comparison of rust infection types produced on nonirradiated (*CK*) and gamma-irradiated seedlings of flax (Bison), wheat (P.I. 94701, Khapli), and oats (Park). With wheat and oats, irradiation preceded inoculation.

Gamma radiation did not noticeably affect the rust reaction of 16 resistant flax varieties that were inoculated before or after application of a 10-kr chronic dose. The reaction of two normally susceptible varieties, Bison and Williston Brown, appeared resistant when inoculation preceded irradiation (Fig. 1). In this case, the fungus renewed growth within 3 days after termination of the exposure. Subsequent experiments with x-rays proved that the inhibitory effect was on the parasite rather than on the host; a 10-kr acute dose was lethal to more than 90 percent of 1-day-old infections.

Table 1. Rust reaction of seedlings of wheat and oat varieties exposed to 6 kr of gamma radiation before inoculation.

Variety	Rust reaction*	
	Control	Irradiated
Wheat inoculated with race 15B†		
St 464 (191365)‡	0;	0;
Khapli (4013)	0; to 1 −	0; to 3
Kenya Farmer (12880)	1	3 to 4
P.I. 94701	3 −	4
Mindum (5296)	4	4 +
Oats inoculated with race 202		
Saia (7010)	0	0
Park (6611)	1	3 to 4
Markton (2053)	4	4
Oats inoculated with race 7A		
Garry (6662)	1	1 to 3
Park (6611)	1	3 ±
Markton (2053)	4	4 +

* Rust reaction class symbols 0, 0;, and 1 denote relative degrees of resistance; symbols 3 and 4 represent moderate and complete susceptibility, respectively; plus and minus signs indicate the upper and lower limits, respectively, of each type.
† For specific assignments of physiologic races 15B, 202, and 7A, see text.
‡ P.I. or C.I. numbers, in parentheses, represent Plant Introduction and Cereal Investigations accession numbers, U.S. Department of Agriculture.

Radiation before inoculation produced no appreciable change in infection type of wheat or oat plants in some resistant varieties, while plants of other varieties became less resistant in varying degrees (Table 1). No case of increased resistance was observed, the trend of change being invariably toward susceptibility. Moderately resistant varieties such as P.I. 94701 appeared particularly amenable to a breakdown of resistance, although a resistant variety such as Kenya Farmer underwent an almost complete change from resistance to susceptibility. The change in reaction on wheat and oats, as contrasted with the inhibitory effect on the growth of flax rust, was clearly due to an alteration of the host and not of the fungus. This was confirmed by the fact that the response occurred whether the full dose preceded inoculation or was divided before and after inoculation. The effect persisted as long as the leaves remained alive.

Chronic irradiation begun 1 day after inoculation was less effective in breaking resistance, probably because such an effect required the revival of fungus growth already stagnated by host-cell necrosis. Approximately 5 kr of chronic gamma radiation, applied at a rate of 1 kr/day, consistently induced distinct changes in the host response. The effective dose range was more readily defined in the acute x-ray treatments. Breakdown of rust resistance could be initiated with as little as 1.5 kr of x-rays and would reach a maximum with approximately 3 kr. The corresponding doses for inhibition of leaf development were 0.5 kr and 1.5 kr; at the latter dose, stunting of the roots and formation of root-tip nodules also became apparent.

Plant injury was not necessarily associated with rust reaction, for some sources of rust resistance, especially in wheat, were not altered, while others responded to varying degrees. This suggests differences in the radiation stability of the physiological processes normally involved in rust reaction, or the interaction of these with other processes not ordinarily related to rust development. A degree of specificity in the nature of the physiological change also is indicated by the difference in the rust-reaction "spectrum" of resistant varieties when tested with more than one physiologic race of the fungus. In wheat varieties that are normally resistant to the widely pathogenic race 15B and the weakly pathogenic race 111, the shift toward susceptibility on irradiated plants was considerably greater with race 15B than with race 111.

Partial-plant irradiation tests revealed the crown—particularly the shoot apex—as the major radiosensitive site. Irradiation of this site was not only responsible for stunting of the leaves but also initiated a portion of the physiological modification favoring rust development on the leaves. Exposure of both roots and crown permitted maximum expression of relative susceptibility, although exposure of the roots alone did not yield decisive changes. Leaf treatments alone produced no injury or significant reduction of resistance.

The physiological basis of the modified rust reaction is not known, although the association of the shoot apex suggests a possible involvement of growth-regulating substances similar to that described for tomato wilt by Waggoner and Dimond (*3*).

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References and Notes

1. H. Hart, *Ann. Rev. Microbiol.* 3, 289 (1949); P. J. Allen, *Ann. Rev. Plant Physiol.* 5, 225 (1954).
2. T. Johnson, *Can. J. Research* C24, 23 (1946); F. R. Forsythe, *Nature* 173, 827 (1954); K. R. Bromfield and C. E. Peet, *Phytopathology* 44, 483 (1954).
3. P. E. Waggoner and A. E. Dimond, *Phytopathology* 46, 125 (1956).
4. This research was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

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