leaves were closed with cellophane tape. A 0.05*M* solution of EMS prepared 2 hours previously was slowly injected into the cotton with a 30 ml-hypodermic syringe until saturation was reached (15 to 30 ml per plant). The cotton was removed 6 hours after injection, together with the partially exposed and presumably less severely treated tassel-branch tips. At flowering the treated pollen containing the dominant genes α β Sh_2 was used to fertilize plants recessive for these markers (a^m sh_2). Mutations or losses of dominant genes through chromosome aberrations occurring before fertilization gave whole endosperm effects, while changes occurring during development result in fractional effects.

The data (Table 1) show that EMS, like x-rays and ultraviolet light, is effective in producing breaks in various regions of the tested chromosome segment. Direct comparisons of total frequencies are impractical because comparable dosage levels of these agents are not known. Ethyl methanesulfonate resembles ultraviolet light in action. Both produce a relatively higher proportion of fractional losses than whole seed losses and both produce losses of β alone as well as an assortment of less discrete changes. In these aspects EMS differs from x-rays, which produce a higher proportion of whole seed losses than fractional losses and which repeatedly fail to produce discrete changes of β alone (*7*).

M. G. NEUFFER
GYULA FICSOR
Department of Field Crops, University of Missouri, Columbia

References and Notes

1. R. E. Heiner, C. F. Konzak, R. A. Nilan, R. R. Legault, *Proc. Natl. Acad Sci. U.S.* **46**, 1215 (1960).
2. A. Loveless and S. Howarth, *Nature* **184**, 1780 (1959).
3. D. M. Green and D. R. Krieg, *Proc. Natl. Acad. Sci. U.S.* **47**, 64 (1961).
4. H. Heslot and R. Ferrary, *Paris, Inst. Natl. Agron. Ann.* **44**, 3 (1958); T. F. Arnason, L. Mohammed, D. Koehler, F. M. Renneberg, *Can. J. Genet. Cytol*, **4**, 172 (1962).
5. P. Brookes and P. D. Lawley, *Biochem. J.* **80**, 496 (1961).
6. M. G. Nuffer, *Genetics* **42**, 273 (1957).
7. Contribution from the Missouri Agricultural Experiment Station; Journal Series 2525.

30 January 1963

Radiation-Induced Gelation of Dilute Aqueous Pectin Solutions

Abstract. *The low-dose gamma irradiation of dilute aqueous pectin solutions after appropriate adjustment of* p*H with hydrochloric or certain other inorganic acids leads to the formation of thermoreversible gels. Maximum gel strength is attained only under optimum conditions of* p*H, concentration, temperature, radiation dose, and exposure time, and only in the absence of oxygen and a number of radiation protectants.*

Pectin, a methyl ester of polygalacturonic acid, undergoes degradation when its aqueous solutions are exposed to ionizing radiation (*1*). No evidence has previously been presented to indicate any radiation-induced coupling reactions between pectin molecules in aqueous solutions. Cross-linking of pectin molecules can be achieved without irradiation by the addition of certain compounds such as formaldehyde, epichlorohydrin, erythrodioxide, and sulfur mustard—a radiomimetric substance (*2*). The cross-linking by bi- or polyfunctional molecules produces a principal-valent gel which is stable, insoluble in water and not thermoreversible. Reversible pectic gels are formed by hydrogen bonding through the addition of acid and a dehydrating agent such as sugar, or by ionic bonding through the addition of a multivalent ion such as Ca^{++}. This report (*3*) describes some conditions under which thermoreversible gel formation can be induced by ionizing radiation without the addition of any dehydrating agent or multivalent ion.

In a preliminary irradiation experiment with a series of dilute aqueous pectin solutions within a wide range of *p*H values, the authors observed gel formation only in those solutions with a *p*H value of about 1.6 to 2.2, and then only at doses of a few hundred kilorads of gamma radiation. For further investigation of this phenomenon, aqueous solutions (2 percent) of a highly refined citrus pectin (NF) with a methoxyl content of 9.8 percent were prepared and adjusted to *p*H 1.0 to 3.0 with HCl.

The samples (in vacuum) were exposed for 90 min at 20°C to 630 krad of gamma radiation, the source of which was Co^{60}. At the end of the irradiation period, the samples were evacuated again to remove any gas bubbles which were entrapped in the gels and which would have interfered with subsequent measurements of gel strength. The viscosity, an indicator of gel strength, was measured with a Brookfield model RVT Synchro-Lectric viscometer and Helipath spindle (T-C). The viscosity measurements in Table 1 were made at 25°C with a spindle speed of 5 rev/min.

All gels which were formed between *p*H 1.4 and 2.6 were reversible; they became dispersed by treatment with mild heat or by exposure to room temperatures for several hours. Maintaining the samples at moderately high temperatures (30 to 50°C) during the irradiation period resulted in weaker gels or no gels at all; the effect was dependent on both the temperature and the length of the exposure period. After irradiation and complete liquefaction of the resulting gels by warming for a few minutes or standing at room temperature for several hours, the viscosity of the liquids thus formed (as measured with an Ostwald-Cannon-Fenske viscometer) continued to decrease over a period of 1 to 3 days. A minimum value equal to that of a solution of the same concentration which had been irradiated with the same dose but without any adjustment of *p*H before irradiation was finally reached.

The strength of the gel formed by a 2-percent pectin solution irradiated at a *p*H of 1.8 increased as the radiation dose increased above about 300 krad until a maximum viscosity was reached at approximately 1 Mrad. Beyond this value there was a decrease in gel strength with additional radiation. At any specific *p*H value, as the pectin concentration increased within the range of 0.5 to 8.0 percent, there were increases in the gel strength at any specific dose, the maximum gel strength attainable, and the dose of maximum gelation, and a decrease in the dose necessary to cause incipient gelation. At concentrations lower than about 0.5 percent, no gel was formed at any radiation dose. Solutions with a pectin concentration higher than 8 percent were too viscous for accurate adjustment of *p*H and evaluation of any gels which might have formed. In all cases of gel formation, there was little change in *p*H and methoxyl content upon irradiation.

Maximum gel strength was attained at a *p*H of approximately 1.8, regardless of the pectin concentration, and also when the *p*H was adjusted with

Table 1. Viscosity of gels formed by irradiation under vacuum of 2 percent aqueous pectin solutions at 20°C after adjustment of *p*H with HCl. The samples were exposed to 630 krad of gamma radiation for a period of 90 minutes. Viscosity measurements were made at 25°C immediately after irradiation.

*p*H	Gel viscosity (10^{-3} cp)	*p*H	Gel viscosity (10^{-3} cp)
1.0	No gel	1.9	144
1.2	No gel	2.0	132
1.4	70	2.2	120
1.6	124	2.4	82
1.7	136	2.6	48
1.8	146	2.8	No gel
		3.0	No gel

certain other inorganic acids such as sulfuric, perchloric, and orthophosphoric acids. On the other hand, there was no evidence of gel formation when the *p*H was adjusted with nitric, hydrobromic, hydroiodic, or hypophosphorous acids or with some organic acids such as acetic and formic acids. Though irradiation in a vacuum resulted in the greatest gel strength, irradiation in an atmosphere of nitrogen or hydrogen was almost as effective in this respect.

Conversely, both oxygen and nitric oxide in sufficiently high concentrations effectively reduced or inhibited gel formation. Gelation was also found to be suppressed by the addition before irradiation, in concentrations of 0.1 to 1.0 percent, of any one of a number of substances, such as AET (S,β-aminoethylisothiouronium·Br·HBr), thiourea, methylene blue, and pyrogallol, all of which had previously been found by the authors (*4*) to act as protectants against the degradation of pectin by irradiation in dilute aqueous solutions. Similarly, the addition of galacturonic acid before irradiation or of irradiation-degraded pectin in concentrations of as low as 0.1 percent reduced gel strength or prevented gel formation, depending on the original concentration of both additive and undegraded pectin. Other substances, such as methylamine hydrochloride, which are poor pectin protectants, had relatively little effect on gel formation even when the concentration was as high as 1.0 percent.

Most of the polysaccharides studied by other investigators undergo degradation when irradiated in the presence of water (*5*). There are recent reports of cross-linking and gel formation in irradiated aqueous solutions of some nonionic cellulose derivatives (*6*). In this laboratory, both dextran and glycogen exhibited an increase in viscosity when irradiated at intermediate moisture levels (about 15 to 90 percent) with low doses of gamma radiation (*4*). This effect, which, in some cases, led eventually to the appearance of an insoluble fraction, was attributed to a coupling reaction between radiation-produced polymer radicals. In the present study, the narrow *p*H range and low dose rates required for gel formation and the adverse effect on gelation of oxygen, nitric oxide, and other free radical scavengers such as AET, strongly suggest that this radiation-induced linking of pectin molecules is related to the production of free radical intermediates. The thermoreversibility of the gel formed indicates the presence of weak linkages such as hydrogen bonds.

I. J. WAHBA
D. F. TALLMAN
L. M. MASSEY, JR.
New York State Agricultural Experiment Station, Cornell University, Geneva

References and Notes

1. Z. I. Kertesz *et al.*, *Radiation Res.* **5**, 372 (1956).
2. H. Deuel, J. Solms, H. Altermatt, Vierteljahrsschr. *Naturforsch. Ges. Zurich* **98**, 49 (1953).
3. New York State Agricultural Experiment Station, Geneva, journal paper No. 1321. Supported in part by research grant AM-02367-05 from the National Institute of Arthritic and Metabolic Diseases.
4. Recent studies conducted by this department. Paper in preparation.
5. F. A. Bovey, *The Effects of Ionizing Radiation on Natural and Synthetic High Polymers* (Interscience, New York, 1958), pp. 189–204.
6. F. C. Leavitt, *J. Polymer Sci.* **45**, 536 (1960); ———, *ibid.* **51**, 349 (1961).

14 December 1962

Density-Gradient Separation of Organic and Inorganic Particles by Centrifugation

Abstract. *Aqueous suspensions of particulate organic and inorganic material were centrifuged in a 2-bromoethanol density gradient. The degree of separation with this new technique is superior to that achieved with sucrose density gradients.*

The separation of organic from inorganic particles is of interest in the study of radioisotope distribution in the particulate matter of natural waters (*1–3*), in attempts to isolate organic material from extraterrestrial sources (*4*), in certain geological studies (*5*), and in criminological investigations (*6*). Density-gradient separation of cell and tissue fractions has been reviewed in several articles (*7*).

Sucrose density gradients, ranging from a minimum of 50 to a maximum of 80 percent sucrose, have been successfully used to separate particulate organic from inorganic material (*1, 2*). Since the 80 percent sucrose used in this type of gradient is difficult to prepare, has a density of only 1.46, and has a rather high viscosity, a search for a better density-gradient system was initiated.

In this investigation 2-bromoethanol (density 1.77, viscosity 4.5 cp) was used. When water is carefully introduced over 2-bromoethanol, a short, steep density gradient forms between the water and the 2-bromoethanol. Test mixtures included a pond-bottom sediment and particulate materials concentrated by continuous flow centrifugation at 28,000*g* (R_{max}, 7 cm) at a flow rate of 150 ml per minute (*1*) from water of the same pond. The organic material of the test mixtures consisted chiefly of bacteria and diatoms with few organic particles larger than 200 μ. All experiments were repeated at least five times.

Approximately 100 ml of an aqueous suspension of the particulate material was carefully layered over 50 ml of dehydrated 2-bromoethanol in a 250-ml centrifuge tube. To eliminate clumping, the particulate material was homogenized before being introduced above the density gradient. The sample was immediately centrifuged for 1 hour, with slow, uniform acceleration, at 1040*g* at 5°C (R_{max}, 23.5 cm; R_{min}, 15 cm).

Aliquots were then removed by aspiration with a tube of 1 mm inside diameter with 180° bend at the tip to minimize disturbance. The aliquots were examined with dark-field phase-contrast microscopy and a counting-chamber technique previously described (*1*).

The viscosity of the 2-bromoethanol was measured in an Ostwald viscometer. Since 2-bromoethanol is toxic, the medial tolerance limit for nutrient broth cultures of *Escherichia coli* was determined by a series of 24-hour bioassays (*8*). The medial tolerance limit for *E. coli* in 2-bromoethanol is 2.5 percent by volume with 24 hours of contact time. Cell counts of *E. coli* made before and after a 2-hour contact period in a mixture of concentrated 2-bromoethanol and water (9 : 1, by vol-