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.

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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-

Table 1. Separation by centrifugation in 2-bromoethanol. For description of the zones see text. The aliquot for zone B was taken from the bottom of the zone. In each case 400 squares were counted and the particles per cubic centimeter were calculated from the counts.

Depth (mm)	Zone	Organic particles	Inorganic particles (10^9/cm^3)
10	A	Few	None noted
40	B	Too dense to count	None noted
45	C	None noted	0.015
50	C	None noted	.023
55	C	None noted	.007
60	C	None noted	.0
65	C	None noted	.038
70	C	None noted	.045
75	C	None noted	.030
80	D	None noted	Too dense to count

ume) showed a reduction of cells from 300×10^6 per milliliter to 291×10^6 per milliliter, a change well within the sampling error.

Visual examination of a centrifuge tube after density-gradient centrifugation showed four distinct zones. Typical results of microscopic examination of the aliquots from various levels in the gradient are listed in Table 1. Virtually no particulate material remained in the clear upper zone (A), which corresponded to the aqueous part of the original particulate suspension. Below, zone B was a dense concentration of organic particles in the gradient formed between the water and the 2-bromoethanol. Below this particulate zone was zone C, nearly devoid of particulate material, which corresponded to the 50 ml of 2-bromoethanol. The bottom band, zone D, below the 2-bromoethanol, was a pellet of concentrated particulate inorganic material. At the lower limits of resolution, differentiation of organic from inorganic particles becomes difficult.

In order to determine further whether particulate organic material could penetrate into the 2-bromoethanol zone of a density gradient, tests were conducted with pure cultures of *E. coli*. The experiments were conducted exactly like the ones described above except that no inorganic material was included in the aqueous particulate suspension. No bacterial cells were present in the 2-bromoethanol zone after centrifugation.

Table 2. Separation by sucrose density-gradient centrifugation. There were 1116 billion organic and 192 billion inorganic particles per cubic centimeter in the uncentrifuged particulate suspension. The 74-percent sucrose zone was used as the separation point of organic from inorganic material. This was centrifuged at 1040*g* for 60 minutes. Each value in columns 2 and 3 was calculated from a count of 80 squares.

Sucrose (%)	Organic particles (10^9/cm^3)	Inorganic particles (10^9/cm^3)
60	7.47	0.525
70	4.08	2.505
74	3.15	3.150
80	1.83	4.005

The best results obtained with a sucrose density gradient are presented in Table 2. In this case 99.7 percent of the organic particles remained in the density zone above the 74-percent sucrose level and 98.4 percent of the inorganic particles were in a zone below the 74-percent sucrose (*1*). The separation obtained with 2-bromoethanol is significantly better, since no organic particles were counted in the 2-bromoethanol at 5 mm below the bottom of the organic pellet, and two orders of magnitude fewer inorganic particles remained suspended in the 2-bromoethanol than were found above the 60-percent sucrose level (*1*).

The isolated organic fraction was removed from the gradient tube by aspiration, after which the remaining material was removed and saved; this was the inorganic fraction of the original sample. The particulate material from both fractions was then isolated from the liquid phase by recentrifugation and aspiration (*1*).

Thus, the use of 2-bromoethanol for density-gradient separation results in very sharp separations of isolated fractions. This system has the advantage of a density so high that no organic particle can pass through it unless associated with an inorganic particle. Such association is precluded as far as possible by homogenization before centrifugation. Further advantages are the low interfacial forces in the water-bromoethanol gradient zone and the relatively low viscosity of the 2-bromoethanol so that no inorganic particles are held back by either interfacial forces or viscosity. Neither an equilibration period nor a gradient engine is required.

This work has concerned particles of microscopic dimensions (0.5 to 200.0 μ); it is obvious that much higher fields would be required for colloidal particles (*9*).

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9. I thank Dr. N. G. Anderson, Biology Division, ORNL, for suggesting a trial of 2-bromoethanol and for his advice and guidance. This work was done as a part of subcontract 2143 from Oak Ridge National Laboratory operated by the Union Carbide Nuclear Corporation for the U.S. Atomic Energy Commission.

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Norepinephrine Synthesis from Tyrosine-C^{14} in Isolated Perfused Guinea Pig Heart

Abstract. *The isolated, perfused guinea pig heart contains all the catalysts required to form norepinephrine from the dietary precursor, tyrosine. The conversion of tyrosine-C^{14} to norepinephrine in the perfused heart occurred at a rate comparable to that estimated for this conversion in vivo. To account for the maintenance of norepinephrine stores in the normal heart, it is not necessary to postulate that the hormone is extracted from the blood.*

Norepinephrine is a normal constituent of the heart, presumably associated with its sympathetic innervation. Although certain extra-adrenal tissues and sympathetic nerves can perform some of the biochemical conversions required for the synthesis of norepinephrine (*1*), the question has never been answered as to the origin of this hormone in the heart. Recent studies on the uptake by the heart of administered radioactive norepinephrine (*2*) give the impression that endogenous stores of cardiac norepinephrine are normally maintained by extraction from the blood. It is conceivable that under certain conditions uptake from the blood may be of some significance. However, the studies reported here show that the heart itself is capable of synthesizing norepinephrine