Pliable, Root-Permeable Layers for Separation of Portions of Experimental Plant Root Systems

Abstract. A description is given of simply made, sturdy, pliable layers which may be placed in rooting media for studies of divided root systems. Roots readily penetrate the layers but form a tight seal in doing so. The layer remains impermeable to water and ions.

In the study of plants under controlled conditions it is often necessary to separate portions of the root system of a growing plant by special layers in the root medium in order to confine ions, water, and microorganisms to the desired regions. In such instances it is frequently desirable to fabricate the layers from materials impermeable to ions, water, and microorganisms but readily penetrable by roots. Such materials have been reported (*1*). Some materials have been cumbersome and have had to be formed in place when needed. Thin layers are desirable but have not always been attained. This report describes a material which possesses required impermeability and which can be penetrated by roots; furthermore, it possesses strength and simplicity of design and forms layers which are reasonably thin.

The layers are composed of a 1:1 mixture (by weight) of paraffin wax and rosin. The substance is prepared by melting and mixing the two components in a common container. When the mixture has reached the boiling point, a quantity (75 to 80 ml, for example) of the liquid is poured on the surface of water which has been heated to 60°C. The water, ½ in. deep, is contained in a shallow pan (a pan 12 in. in diameter and 1½ in. deep has sufficed for this purpose). Water of this depth seems to exhibit a stability of surface desirable for preparation of the layers. The poured mixture is allowed to cool until firm.

The material, prepared according to these specifications of quantity of mixture, temperature, and size of pan, produces a layer about 11 in. in diameter and about 1 mm thick. The layer is of rather uniform thickness throughout, thinning out somewhat at the very edge. The thickness obtained is primarily a function of the initial temperature of the water: lower temperatures produce thicker layers. The best results were obtained by pouring the melt down a glass rod which was in contact with the water at one end. This eliminated the formation of bubbles in the layer. What temperature produces a layer of a given thickness depends upon the composition of the paraffin and rosin and is best determined by experimentation.

The cooled layer can be removed from the pan by hand, molded, cut, handled, and pressed into shape to conform to a vessel, then sealed into place with a piece of heated metal or ceramic. A soldering iron has sufficed for this purpose. If the material is accidentally torn, repair by the same procedures is possible. When used in a pot experiment, the material showed no evidence of decomposition over a 3-month period. It appeared to have no toxic or inhibitory effect on the roots of either white clover (*Trifolium repens* L.) or common Bermuda grass (*Cynodon dactylon* L.).

The material appears to be impermeable to ions. In a study in which radioisotopes of strontium were used, no movement of the ions through layers of the wax material was observed. The material is impermeable to water. It appears, further, that a tight seal exists between the material and a root which has penetrated the layer. Penetration of roots has not produced measurable movement of water or ions past the layer.

JOHN F. STONE
JAMES R. MULKEY, JR.
Department of Agronomy, Oklahoma State University, Stillwater

References

1. A. H. Hendrickson and F. J. Veihmeyer, *Plant Physiol.* **6**, 567 (1931); A. S. Hunter and O. J. Kelley, *Soil Sci.* **62**, 441 (1946); H. J. Mederski and J. H. Wilson, *Soil Sci. Soc. Am. Proc.* **24**, 149 (1960); J. E. Weaver, F. C. Jean, J. W. Crist, "Development and Activities of Roots of Crop Plants," *Carnegie Inst. Wash. Publ. No. 316* (1922).

16 September 1960

Effect of Mitomycin C on Lateral Root-Tip Chromosomes of Vicia faba

Abstract. Lateral root-tip chromosomes of *Vicia faba* were treated with mitomycin C. Chromatid aberrations were observed from 24 through 96 hours after treatment. The effect of mitomycin C was not altered appreciably by changes in *p*H, temperature, oxygen tension, or lack of adenosine triphosphate. The relationship of mitomycin C to other radiomimetic compounds is discussed.

In two recent papers Sekiguchi and Takagi (*1, 2*) demonstrated two remarkable effects of mitocycin C. They showed an inhibition of deoxyribonucleic acid synthesis by phage-infected *Escherichia coli* in the presence of mitomycin C (*1*) and the production of what the authors termed "abnormal DNA" by bacteriophages infecting *E. coli* in the presence of a high level of mitomycin C (*2*). Sekiguchi and Takagi also observed that, although DNA synthesis was inhibited in *E. coli* during treatment with mitomycin C, there was

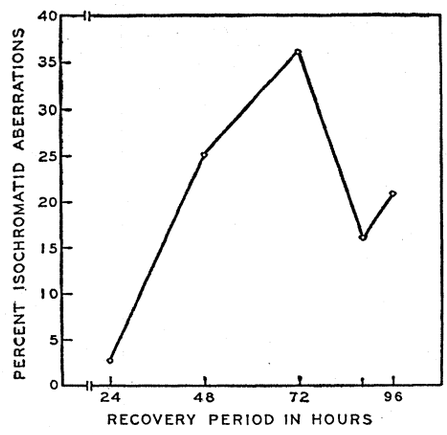

Fig. 1. Frequencies of isochromatid aberrations obtained at different fixation times after treatment.

no such inhibition of protein or ribonucleic acid synthesis (*1*). It seemed, then, that mitomycin C would be an excellent agent to use in determining whether the "rejoining system" would be inhibited in the absence of DNA synthesis, as it has been shown to be in the absence of protein synthesis (*3*). During tests to determine whether rejoining was in fact inhibited by mitomycin C, chromosomal aberrations were observed in root-tip cells treated with the agent alone. This is a report of the ensuing study of mitomycin C as a member of a rapidly increasing list of interesting radiomimetic compounds.

The material used in the experiments consisted of lateral root tips of the broad bean, *Vicia faba* (*4*). As has been previously described (*5*), the roots were grown in shell vials in the dark at 25°C for 7 days after an initial 24-hour period of soaking. The tap water was changed daily during growth and recovery (the latter being the time interval between treatment and fixation). Twenty-four hours before treatment the roots were placed in an incubator at 17°C. Treatments were carried out at 20°C, unless otherwise stated, and recovery took place at 17°C in the dark. The root tips were treated with 0.05-percent colchicine for 3 to 4 hours before fixation in cold alcohol and acetic acid (3:1), and slides were prepared as Feulgen squashes. The treatments were carried out in shell vials. The method for the introduction of gases has been described previously (*6*). Pretreatment with dinitrophenol (DNP) for 2 hours was carried out to test the effect of energy deprivation on the mitomycin C effect (*7*).

Various concentrations of mitomycin C were tried. It was found that concentrations of 0.1 percent, 0.01 percent for 1 hour, and 0.001 percent for 2 hours were lethal, death of cells ocur-

Table 1. Influence of *p*H, oxygen tension, dinitrophenol (DNP), and temperature on the effect of mitomycin C on root-tip cells of *Vicia faba*. All roots were treated with 0.001 percent mitomycin C; the recovery period was 72 hours; 200 cells were scored for each treatment.

Treatment conditions	Normal (%)	Abnormal (%)	Deletions (%)	Isochromatids (%)	Exchanges (%)
Influence of pH; *roots treated at 20°C in air*					
*p*H 8.5	70	30	0	32	21
*p*H 6.8	66	34	0	36	17
*p*H 4.5	62	38	0	55	15
Influence of oxygen tension; roots treated at pH *6.8 at 20°C*					
0% O_2 (N_2)	54	46	0	39	21
20% O_2 (air)	55	45	0	56	16
100% O_2	58	42	0	44	19
Influence of 1×10^{-4} M *dinitrophenol; roots treated at* pH *6.8 at 20°C in air*					
DNP present	55	45	0	58	16
DNP absent	58	42	0	36	16
Influence of temperature; roots treated at pH *6.8 in air*					
Temp. 10°C	52	48	0	61	15
Temp. 25°C	55	45	0	56	16

ring within 48 hours. A concentration of 0.0005 percent for 1 hour produced no lethal effect, some inhibition of mitosis (few division figures were apparent 24 hours after treatment), and a low frequency of chromatid aberrations 48 and 72 hours after treatment. A 0.001-percent concentration of mitomycin C with a 1-hour treatment period proved to be the most efficient combination for examining the effect of this compound on chromosomes. After treatment with 0.001-percent mitomycin C there was a marked inhibition of mitosis. Few division figures or aberrations occurred 24 hours after treatment. The graph (Fig. 1) illustrates the frequencies of isochromatid aberrations obtained at different fixation times after treatment. The highest frequency was obtained at 72 hours and the frequency decreased thereafter, but the level of isochromatid aberrations was still high 96 hours after treatment. It would be very difficult to determine the mitotic stage that is most sensitive to mitomycin C, since mitotis is inhibited to such an extent after treatment. The highest frequency of aberrations coincided with the highest frequency of division (at 72 hours), but the rate was maintained at a constantly high level from 48 through 96 hours. These data suggest that at least most interphase stages are sensitive to mitomycin C.

Since many of the chemical mutagens produce different results in different environments, it was of interest to determine how such alterations of the environment as *p*H changes, variations in oxygen tension, elimination of adenosine triphosphate, and changes in temperature altered the effect of mitomycin C. The results of experiments designed to determine the nature of the influence of these environmental changes on the effects of mitomycin C are listed in Table 1. The results seem to indicate that varying the environment during treatment with mitomycin C does not appreciably alter the ability of this mutagen to induce breaks.

Another interesting property of many radiomimetic compounds is their ability to produce breaks in one or more specific areas along the lengths of the chromosomes of *Vicia faba*. In contrast to breaks induced by x-rays, which seem to be distributed more or less at random (*8*), breaks induced by 8-ethoxycaffeine are concentrated in the nucleolar constriction (*6, 9*), those induced by maleic hydrazide occur in segments of heterochromatin on either side of the centromere of the long chromosome, and breaks induced by beta-propiolactone, nitrogen mustard, di(2,3-epoxypropyl) ether, and potassium cyanide occur in heterochromatic segments in the middle of the short chromosomes (*8, 10, 11*). Also, when breaks are distributed in somewhat random fashion, the rates obtained by dividing the total number of breaks in the short chromosomes by the total number of breaks in the long chromosomes (*S/L* ratio) is approximately 2.5 (*11*). The *S/L* ratios observed after treatment with 8-ethoxycaffeine or maleic hydrazide are less than 1 (*11*), whereas those observed after treatment with nitrogen mustard or di-epoxide are greater than 2.5 (*11*).

Over 800 breaks (in 450 cells) induced by mitomycin C were scored as to location. The *S/L* ratio was observed to be about 1.5, indicating some specificity for the long chromosomes. Approximately 80 percent of the breaks observed in the long chromosomes were situated in the nucleolar organizer constriction, about 15 percent being located in the heterochromatic segments on either side of the centromere. Most of the other breaks occurred in the heterochromatic segments of the short chromosomes. In all, about 93 percent of the breaks were located in heterochromatin.

Mitomycin C, like most radiomimetic compounds, has unique qualities. For example, like beta-propiolactone, nitrogen mustard, di-epoxide, and several others, it seems to act independently of oxygen tension, temperature, and *p*H; but the breaks induced are mainly concentrated in the long chromosomes as are those induced by 8-ethoxycaffeine and maleic hydrazide, which, however, are known to exhibit a strong oxygen and temperature dependency.

Because mitomycin C is of interest as a carcinostat, it is desirable to know whether its anticarcinogenic characteristics can be maintained through its action on DNA synthesis at the same time that its capacity to break chromosomes is inhibited; conceivably, its inherent toxicity to human beings would be reduced by such inhibitions. It is of interest, therefore, to know to what degree its action on DNA synthesis is related to its ability to break chromosomes, and to what degree both properties are related to its action as an anticarcinogen (*12*).

TIMOTHY MERZ
Department of Biology, Johns Hopkins University, Baltimore, Maryland

References and Notes

1. M. Sekiguchi and Y. Takagi, *Nature* **184**, 1134 (1959).
2. ———, *Virology* **10**, 160 (1960).
3. S. Wolff, *Am. Naturalist* **94**, 85 (1959).
4. The seeds (variety Seville Long Pod) were obtained from Carter's Tested Seeds Ltd., London.
5. T. Merz, *J. Biophys. Biochem. Cytol.* **5**, 135 (1959).
6. B. A. Kihlman, *Hereditas* **41**, 384 (1955).
7. Mitomycin C was obtained through the courtesy of Dr. R. D. Coghill of the National Cancer Institute, National Institutes of Health, Bethesda, Md.
8. C. E. Ford, *Hereditas* **35**, suppl., 570 (1949); S. H. Revell, *Heredity* **6**, suppl., 107 (1953).
9. B. A. Kihlman and A. Levan, *Hereditas* **37**, 382 (1951).
10. C. P. Swanson and T. Merz, *Science* **129**, 1364 (1959).
11. B. A. Kihlman, *J. Biophys. Biochem. Cytol.* **3**, 363 (1957).
12. This work was supported in part by the American Cancer Society and the National Institutes of Health.

15 July 1960

Sensory Deprivation and Pain Thresholds

Abstract. Four days of sensory deprivation produced a significant lowering of thresholds for electrically induced pain.

Eighteen male adult subjects served in a study to determine the effects of sensory deprivation upon cutaneous pain thresholds (*1*). Each subject spent a minimum of 1 hour per day in the deprivation chamber, during which time pain thresholds were measured. These periods were considered to be practice periods. Most subjects served in this manner for 3 or 4 days before their thresholds became sufficiently stable to