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The Adaptability of Several Histological Techniques to the Preparation of P32 Radioautographs from Plant Tissues

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With the increased use of radioactive isotopes in plant research, there has been a corresponding increase in the application of radioautographic techniques. Through the use of such radioautographs, tissue localization of the radioactive elements has been accomplished thereby giving additional insight into the accumulation and role of various elements in specific tissues.

One of the radioactive elements that has found widespread application in physiological research is P^{32}. Although a number of workers have prepared P^{32} autographs from unsectioned plant tissues, very few have been made using histological sections (McIlrath, 1948). Because of the widespread use of this radioactive element in plant research and the very few histological radioautographs found in the literature, this problem was undertaken to determine which of several histological techniques might be adaptable to the preparation of such radioautographs.

Methods

The problem of the suitability of various histological methods for the preparation of P^{32} radioautographs was approached in this study in an indirect manner. Rather than making direct determination of the loss of radioactive phosphorus caused by the various techniques, the leaching of the normal phosphorus content of the tissues was determined. From these results prediction as to the adaptability of the methods for use with P^{32} is made. It should be pointed out, however, that one factor which is not taken into account in such an approach is whether or not the fixation and dehydration procedures interfere with the radioactivity of the isotope (McIlrath, 1948).

The material selected for use in this study was the stems of forty-two day old sunflower plants which had been grown in the greenhouse. On a dry weight basis, the total phosphorus content of these stems was 0.456 per cent. The stems were cut in lengths of approximately one-fourth of an inch before being carried through the various procedures.

Stem tissue was sectioned on the freezing microtome by freezing it in a matrix of fifteen per cent aqueous gum arabic solution. This

tissue was not soaked in the gum arabic solution prior to sectioning. Carbon dioxide gas was employed in the freezing process.

One set of stem sections was killed and fixed in formalin-aceto-alcohol, dehydrated in a normal butyl alcohol series and embedded in 52-54°C m.p. paraffin (Fisher Tissuemat) (Johansen, 1940; Sass, 1940). Another set was killed, fixed and dehydrated in Carnoy's fluid and embedded in paraffin (Johansen, 1940; Sass, 1940). A third group was fixed in formalin-aceto-alcohol and embedded in carbowax according to a method devised by Van Horne (1949). In these last three procedures the tissues were aspirated when placed in the killing and fixing solutions. These techniques will henceforth be referred to in this paper as the FAA, Carnoy and Carbowax I methods respectively.

Because of the relatively long time taken by the before mentioned embedding methods, two additional procedures requiring less time were also employed. The first was a freezing-drying method recommended by Leblond (1943) for animal tissue and the second a modification of Van Horne's (1949) carbowax technique. These two methods will be referred to as the Leblond and Carbowax II methods. In the Leblond method the stem sections were cut in one-sixteenth inch lengths to alleviate longitudinal splitting when they were dropped in the acetone dry ice mixture for quick freezing.

The time schedule used in carrying the stem material through the various steps in the above methods is recorded in Table 1. Sam-

Table 1

The schedule in hours for the procedure followed in the various histological methods.

Series	Killing & Fixing	Dehydration	Embedding	Total Hours
FAA	8	9	8	25
Carnoy	3	——————→	8	11
Carbowax I	8	0	5	13
Leblond	0	3	1	4
Carbowax II	0	0	5	5

plings of plant material for chemical analysis of the residual phosphorus were taken at the end of each time period recorded in this table. These analyses were made according to the method of Wolf and Ichisaka (1947). Tissues from all embedded series were sectioned on the rotary microtome to determine their suitability for microscopic study.

Data and Discussion

It is known that the largest percentage of the phosphorus in plants is in a water soluble form (Miller, 1938). In the sunflower stems used in this study, the soluble phosphorus content constituted over seventy per cent of the total amount present. With such a high soluble phosphorus content, if localization of the total P^{32} present in the tissues is to be accomplished, a histological method causing little or no leaching of phosphorus must be adopted. A second requisite of the procedure used must be that it yields material of satisfactory quality for microscopic study. Another factor of lesser importance which one may wish to take into consideration is the time involved in the histological technique. This factor may become increasingly more important if several half lives of the P^{32} are allowed to pass between administration of the phosphorus and harvesting of the plants for the preparation of the autographs. Such a time interval may be necessary if one desires that some of the radioactive phosphorus be metabolized and fixed in an insoluble form in the new plant growth.

Freezing

Of the various methods checked, the freezing technique involved the least procedure time and resulted in no detectable loss of phosphorus (Table 2). Satisfactory sections were obtained with this method without difficulty if they were cut at approximately twenty-five microns. Sections somewhat thinner than this were obtained with difficulty, although they were inferior in quality. The limitation of a minimum thickness at which sections can be cut by this procedure may be a serious disadvantage if precise localization of the radioactive element is to be accomplished. Evans (1948) states that sections should not be over five to ten microns if maximum resolution in the radioautograph is to be obtained. Another factor inherent in this method which tends to give poor resolution in the radioautograph is that, since the material is not dehydrated, some medium must be placed between the section and the photographic emulsion. In spite of these limitations, the fact that little or no phosphorus is lost from the sections makes it an acceptable method for the localization of general areas of radioactive phosphorus concentration.

FAA

The FAA method yielded very satisfactory histological sections, and the stem material prepared in this manner could be cut at five microns without difficulty. The microtome compression encoun-

tered when cutting sections at this thickness (Shields & Dean, 1949) should not offer any serious handicap in the preparation of radioautographs.

This method resulted in a loss of a large portion of the total phosphorus of the embedded stem segments and therefore is not practical for the observation of total phosphorus (Table 2). It may, however, have some adaptation in the localization of chemically combined phosphorus. This statement is made in view of the fact that the amount of phosphorus present in the embedded material closely approximated the insoluble phosphorus content of the untreated sunflower stems.

Table 2

Percentage of the total phosphorus content of sunflower stems lost after various steps in the histological procedures.

Freezing	FAA			Carnoy		Leblond
Cut Section	Killing & Fixing	Dehydration	Embedding	Fixing & Dehydration	Embedding	Embedding
0.0	32.9	61.4	71.4	46.4	57.6	0.0

Carnoy

Histological sections prepared from the stems fixed and dehydrated in Carnoy's fluid, according to the schedule previously given, were very poor quality. A large percentage of all cells except those of the xylem elements and the mechanical tissue were collapsed. Limited infiltration of paraffin took place in the embedding process with the result that the material was extremely difficult to section. No sections were obtained less than fifteen microns in thickness. This method as outlined was therefore an unsatisfactory histological technique for material with such bulk and high degree of hydration as is found in tissues such as the sunflower stem. From the standpoint of phosphorus leached, this method was also unsatisfactory (Table 2).

Leblond

Although no detectable quantity of phosphorus was leached in the Leblond method (Table 2), it was an unsatisfactory histological procedure for sunflower stem tissue. With this method there seemed to be a very limited paraffin infiltration as indicated by the high percentage of cells collapsed. The minimum thickness at which the embedded material could be sectioned was twenty-five microns.

Carbowax

In that the carbowax used in this experiment had been polymerized with phosphoric acid, it was impossible to determine phosphorus loss by analysis of the embedded tissue. Indirect determinations were made by placing stem sections in distilled water and allowing them to stand for five hours at a temperature equivalent to that used in the carbowax embedding. During this time 45.4% of the total phosphorus was leached from the stems. Carbowax being water soluble, one could probably assume that the phosphorus leached during the embedding process in the Carbowax II method would be close to this magnitude. The formalin-aceto-alcohol leaches 32.9% of the total tissue phosphorus in the Carbowax I method, hence it is likely that a large portion, if not all, of the remaining soluble phosphorus is lost in the embedding process. This being the case, one might assume, as was done in the FAA method, that the Carbowax I method could be adapted to the determination of chemically bound phosphorus. The Carbowax I method, however, yielded histological sections which were inferior to the FAA method. It has the further disadvantage that the radioautographic procedures that can be utilized are limited by the water soluble nature of the carbowax. The Carbowax II method was entirely unsatisfactory from a histological standpoint. In this material the collapsed cortical cells tended to tear away from the remainder of the stem tissue in the microtoming.

Summary

Of the various histological procedures checked, the freezing microtome method was the only one yielding satisfactory sections without excessive loss of phosphorus. The FAA method gave excellent histological sections but caused excessive leaching of the stem's phosphorus content. This loss, however, closely approximated the soluble phosphorus content of the stems; thus this method may have some application in the localization of combined phosphorus of the tissues.

The Carnoy, Leblond and Carbowax methods as outlined were all unsatisfactory procedures for use in the preparation of radioautographs. The Carnoy and Carbowax I method caused excessive leaching of phosphorus and the Carnoy, Leblond and Carbowax II methods did not give satisfactory histological sections.

Literature Cited

Evans, T. C. 1948. Selection of radioautographic technique for problems in biology. Nucleonics 2:52-58.

Johansen, Donald Alexander. 1940. Plant microtechnique. McGraw-Hill Book Company, New York.

Leblond, C. P. 1943. Locating I in tissues autographically. Stain Tech. 18:159-164.

McIlrath, Wayne J. 1948. Radioautographic technique. Unpublished. Dept. of Botany, State University of Iowa. 44 pp.

Miller, Edwin C. 1938. Plant physiology. McGraw-Hill Book Company, New York.

Sass, John E. 1940. Elements of botanical microtechnique. McGraw-Hill Book Company, New York.

Shields, Lora Mangum and H. L. Dean. 1949. Microtome compression in plant tissue. Am. Jour. Bot. 36:408-416.

Van Horne, R. L. 1949. Water soluble embedding materials for botanical microtechnique. Jour. Am. Pharm. Assoc. In press.

Wolf, Benjamin and Vernon Ichisaka. 1947. Rapid chemical soil and plant tests. Soil Science 64:227-244.

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