

This method of sampling provides a means of qualitatively assessing the microbiological material suspended in the air in any situation. It should be especially useful in hospitals and in the field of agriculture (3).

D. C. O'CONNELL

N. J. B. WIGGIN*

G. F. PIKE†

Defence Research Kingston Laboratory,
Barriefield, Ontario, Canada

References and Notes

1. L. J. Goldberg, H. M. S. Watkins, E. E. Boerke, M. A. Chatigny, *Am. J. Hyg.* **68**, 85 (1958).
2. D. W. Henderson, *J. Hyg.* **50**, 53 (1952).
3. This report is a contribution from the Defence Research Board, Kingston, Ontario, Canada (DRKL report No. 88 A).
4. R. W. Reed and G. B. Reed, *Can. J. Research* **E26**, 317 (1948).
- * Present address, Defence Research Medical Laboratory, Downsview, Ontario, Canada.
- † Present address, Defence Research Chemical Laboratories, Ottawa, Ontario, Canada.

4 December 1959

Incorporation of Tritiated Thymidine into Nuclei of Shoot Apical Meristems

Abstract. Tritiated thymidine enters readily into certain excised plant parts and into small aquatic plants. Attempts to introduce the radioisotope into shoot tips of seed plants via the roots have not proved satisfactory. The label readily enters the shoot if applied directly to immature leaves of a bud after the application of a wetting agent.

The general availability of tritiated thymidine has resulted in its use on a variety of organisms. Its use is designed to elucidate specific biochemical pathways of deoxyribonucleic acid synthesis which do not yield themselves readily to chemical analysis. Methods of application have varied considerably, as follows: small animals have been injected

(1) or fed (2) with success; excised portions of tobacco pith incorporate the label (3); roots readily absorb thymidine (4); algae and aquatic cryptogams absorb the radioisotope with particular facility (5). Attempts to introduce and bring about incorporation of H^3 -thymidine into the shoot systems of intact seed plants have not always been successful.

In a preliminary experiment, young (6 to 7 in. high) intact plants and excised shoots of *Chenopodium album* were placed in half-strength Hoagland's solution and H^3 -thymidine ($10 \mu\text{C}/\text{ml}$). Shoot-tip samples were fixed at 3- and 5-day intervals. The material was fixed in FPA, processed in butyl alcohol, embedded in paraffin, and sectioned at 7μ ; the sections were then covered with autoradiographic stripping film AR.10 (Kodak). After exposure for 14 days the film was developed in DK19b (Kodak); the sections were stained with Harris' hematoxylin, and the slide was made permanent by mounting the sections and superposed film in Harleco resin. Examination of the autoradiographs did not reveal the presence of the label in the shoot apical regions, but nuclei of the root tips were labeled. Although longer periods of exposure to thymidine were not tried, the results of the preliminary tests indicated that the isotope becomes fixed in the meristematic root tips but does not move readily in the transpiration stream, as does P^{32} for example. The results indicated that other modes of movement and transport must be involved.

The application of H^3 -thymidine "dropwise" to the terminal bud [preceded by the application of a drop of the wetting agent Tween-20 (0.1 percent)] resulted in foliar penetration and subsequent movement of the label to all young leaves, to the shoot apex, and to subjacent stem regions. One drop

(approximately 0.05 ml of a solution containing $10 \mu\text{C}$ of the label per milliliter) was applied each day for 3 days. Whether movement, after initial penetration, was primarily through the phloem was not determined. To test the possible rapid movement in the phloem, the label was applied to fully expanded photosynthesizing leaves. After 3 days very little, if any, of the isotope could be detected in the autoradiographs of meristematic regions. It is possible that the thymidine molecule did not penetrate the mature outer walls of epidermal cells. The radioisotope was introduced also by injecting it into the stem a short distance below the shoot apex. This procedure resulted in the general distribution of the label into young leaves and the shoot apex. This method, however, does not appear to be as effective as that of tip application.

As may be noted in Fig. 1A, labeled nuclei are apparent (they appear totally black at this magnification) in the leaf to the left, in the developing pith, and to a lesser extent in the leaf at the right. Presumably the label was initially in direct contact with the leaf to the left. In Fig. 1B it may be noted that nuclei of cells of the shoot apex near the tip (on the right), as well as those in subjacent regions, have reduced silver grains over them. The two centers of activity on the upper right flank are well within the region of presumed mitotic inactivity, as described by some workers (6). It would not appear that these cells are inactive in deoxyribonucleic acid synthesis. If endomitotic reduplication and metabolic turnover are ruled out, the incorporation of thymidine into deoxyribonucleic acid is indicative of subsequent mitotic activity.

The utilization of H^3 -thymidine, in conjunction with the use of P^{32} (7), should provide reliable information relative to sites of mitotic activity and aid in the elucidation of the growth of shoot apical meristems (8).

ERNEST M. GIFFORD, JR.

Department of Botany,
University of California, Davis

References and Notes

1. A. Lima-de-Faria, *Science* **130**, 503 (1959).
2. R. C. King and R. G. Burnett, *ibid.* **129**, 1674 (1959).
3. N. K. Das, K. Patau, F. Skoog, *Chromosoma* **9**, 606 (1957-1958).
4. J. H. Taylor, P. S. Woods, W. L. Hughes, *Proc. Natl. Acad. Sci. U.S.A.* **43**, 122 (1957).
5. C. R. Stocking and E. M. Gifford, Jr., *Biochemical and Biophysical Research Communications* **1**, 159 (1959).
6. R. Buvat, *Ann. sci. nat. Bot. et biol. végétale* **13** (1951), 199 (1952); *Ann. Biol.* **31**, 595 (1955).
7. C. R. Partanen and E. M. Gifford, Jr., *Nature* **182**, 1747 (1958).
8. This work was supported by the National Science Foundation (research grant G-6130). The labeled thymidine was supplied by Schwarz Laboratories.

25 September 1959

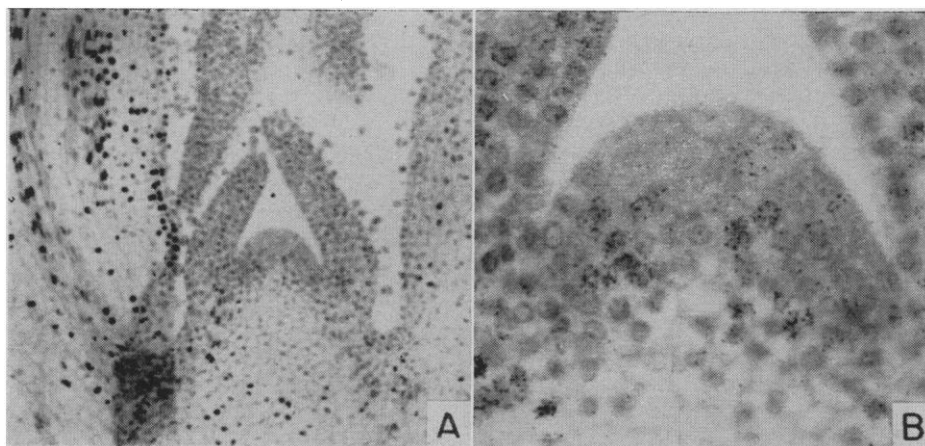


Fig. 1. (A) Shoot apex and young leaves of a bud of *Chenopodium album* treated with H^3 -thymidine. Nuclei which incorporated the label are dark in appearance ($\times 125$). (B) Details, shoot apex of same specimen ($\times 580$).