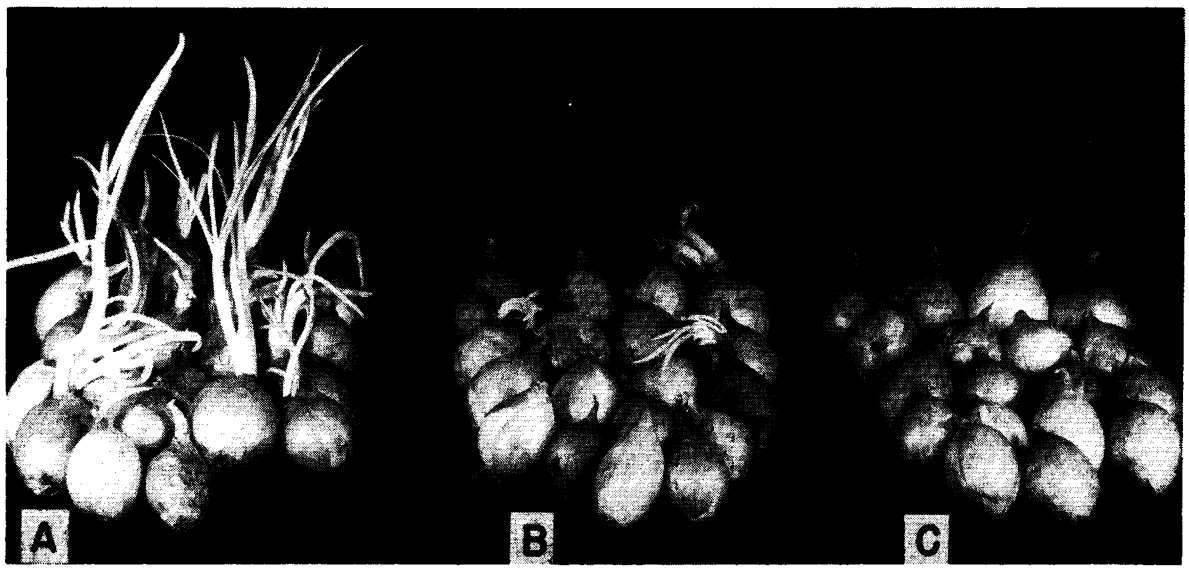


FIG. 1. The effects of preharvest foliage sprays of maleic hydrazide on sprouting of sweet Spanish onions. A, controls (nontreated); B, maleic hydrazide, 500 ppm; C, maleic hydrazide, 2,500 ppm.

(Table 1) was also observed. Gross longitudinal sections of bulbs resulting from treatment with 2,500 ppm of maleic hydrazide revealed an internal structure that was normal and indistinguishable from nonsprouting controls (not treated). Flavor, color, and odor were apparently not affected. Some of the bulbs from plants that had received the 500 and 2,500 ppm of maleic hydrazide were held for an additional 6 weeks in storage at 55° F and photographed April 15 (Fig. 1). Similar lots of bulbs resulting from treatment with 2,500 ppm of maleic hydrazide and planted March 5 in the greenhouse remained sound but completely dormant for 8 weeks, whereas nontreated bulbs grew normally, producing profuse roots and large vegetative tops. Other chemicals caused no inhibition of sprouting. The sodium salt of α-naphthaleneacetic acid and 2,4,5-trichlorophenoxyacetic acid resulted in a significantly greater weight of sprouts, and the "Barsprout" formulation increased significantly the percentage of storage loss from breakdown (Table 1).

Some of the inhibiting effects of maleic hydrazide on plant growth have recently been described by Schoene and Hoffman (*3*), and subsequent reports (*4, 5, 6, 7, 8*) suggest that it has unique properties as a regulator of plant development. Results similar to those described herein for onions have been obtained with carrots, and studies are being conducted with other commonly stored root crops, sugar beets, and potatoes.

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## Loss of Choline Esterase Activity in Nerve Tissue Resulting from Processes of Histological Preparation[1]

**W. L. Hard and Merle D. Fox**

*Department of Anatomy, School of Medicine, University of South Dakota, Vermillion*

In the course of research involving studies of choline esterase activity in dog tissues, it became of interest to determine quantitatively how much of the enzyme activity present in fresh tissues remains in the paraffin-embedded tissues as employed in the histochemical technique (*1*).

For this purpose the entire cord and medulla of an adult dog were divided linearly into 48 approximately equal segments, weighed to the nearest 0.1 mg. Successive segments were then treated as follows: (*a*) the first segment was used to demonstrate the total enzyme activity present; (*b*) the second, maintained at refrigerated temperatures, was fixed in 95% alcohol for 6 hr, dehydrated in absolute alcohol for 12 hr, placed in a mixture of 1 part absolute alcohol and 1 part benzene for 30 min; (*c*) the third segment was treated as in (*b*) but in addition was immersed in melted paraffin at 52° C for 2 hr and deparaffinized in xylene for 1 hr; (*d*) the fourth segment was embedded, sectioned, and prepared by histochemical methods (*1*).

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TABLE 1

| Hydrolysis in fresh tissue | To paraffin | | Through paraffin | |
|---|---|---|---|---|
| | Hydrolysis | % lost | Hydrolysis | % lost |
| 0.915 (1.68–0.37) | 0.54 (0.97–0.24) | 38.8 | 0.22 (0.38–0.08) | 76.0 |

The tissue blocks subjected to the various steps of fixation and embedding were then homogenized and extracted with 30% glycerine. The residual enzyme was determined by the microtitrametric method of Glick (*2*), using acetylcholine as the substrate in a concentration of 0.51%. Total hydrolysis was determined on 3 separate aliquots of extract from each tissue segment, yielding 39 determinations for each group of treated segments, *a*, *b*, and *c*. These data have been summarized (Table 1) by averaging all determinations, but the ranges are indicated by the bracketed figures. The highest values were obtained for the medulla and thence, in descending order, the lumbosacral enlargement, cervical, and thoracic cord. The standard error for all determintions proved to be 0.042. It may be noted here that extracts of nerve tissue and hemolyzed red cells hydrolyzed both myristoyl choline, as used in the histochemical technique (*1*), and acetylcholine in an essentially similar graphical configuration at varying but equivalent substrate concentrations. Thus, peak activities for both substrates were obtained at similar concentrations, and a parallel reduction in activity because of higher concentrations occurred for the 2 substrates. However, the total hydrolysis of myristoyl choline was only 54.2% that obtained for acetylcholine. We have felt justified in using the latter substrate to take advantage of this heightened total hydrolysis and reduce the potential error accordingly. The units of enzyme activity are expressed in μl of 0.1*N* NaOH/mg of tissue. For purposes of comparison, the enzyme concentration determined for the fresh, untreated segments was taken as 100% activity, and the loss expressed as the per cent activity remaining.

The process of fixation and dehydration destroys or inactivates about 38% of the enzyme present (Table 1), and paraffin embedding reduces enzyme activity an additional 38%. Thus three-fourths of the normal tissue enzyme activity is destroyed as a result of the rigors of fixation, dehydration, and embedding. This figure is not unlike previous reports of the loss of non-specific esterases (*3*) and phosphatases (*4*), wherein 60–95% loss in the respective enzymes was encountered in paraffin-embedded tissues. It would thus seem that consistently high enzyme losses for the esterases are to be expected in the preparation of paraffin-embedded tissues. Since the process of embedding is primarily concerned with the chemically inert substance paraffin, it is assumed that the major loss is due to the relatively high temperatures encountered. It may be recalled (*5*) that choline esterase is quite thermolabile, being rapidly inactivated at temperatures approaching 60° C.

Noting the high loss of enzyme activity in paraffin-embedded tissues, we found it possible to ascertain roughly the lower limits of enzyme concentration that must remain in the tissue in order to secure a positive histochemical reaction. In a series of experiments to be reported elsewhere, the cervical sympathetic cord was transected and the choline esterase activity in the superior ganglion determined by both histochemical and quantitative methods. Within 3 days postoperatively the histochemical reactions were negative, presumably because of loss of the enzyme in the degenerated terminal endings of the preganglionic neurons. However, quantitative determinations on these ganglia showed only a 10–15% loss of enzyme. To explain the negative histochemical results, it is presumed that the initial loss of enzyme (10%), together with the technical loss in preparing the sections (70%), results in some 20% of residual activity remaining as compared with the normal tissue, an amount insufficient to accommodate the necessary hydrolysis of the substrate to permit histochemical localization. There are undoubtedly other factors that further serve to limit the sensitivity of this reaction, but certainly the above evidence suggests that a negative histochemical reaction does not necessarily mean an absence of the enzyme, and, correspondingly, due caution should be exercised in interpreting this type of preparation. The value of making simultaneous quantitative studies, wherever the nature of the tissue elements permits, is self-evident.

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## A Technique for Whole Mount Autoradiographs of Rabbit Mammary Glands[1,2]


**Walter S. Lundahl, Joseph Meites, and L. F. Wolterink**

***Department of Physiology and Pharmacology, Michigan State College, East Lansing***


We have observed that growing mammary glands of rabbits and rats readily take up radioactive phosphorus ($P^{32}$). By the use of the autoradiographic technique described here, the extent of mammary proliferation, as well as the regions of most active growth, can be clearly differentiated.

Autoradiographs of the mammary glands of male and female rabbits were prepared by the following procedure:


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