PRECAUTIONS IN THE USE OF LANOLIN AS AN ASSAY DILUENT FOR PLANT GROWTH SUBSTANCES

C. T. Redemann, S. H. Wittwer, and H. M. Sell

(WITH ONE FIGURE)

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Lanolin is frequently used as a diluent for applying growth substances to plants. Its earliest use was described by Laibach (1). He applied unilaterally, to the first internodes of Phaseolus multiflorus seedlings, a lanolin paste containing the test substance. The presence of growth substances induced negative stem curvatures. Subsequently, Laibach and co-workers (2, 3) applied lanolin pastes containing dissolved ether-extractible materials of urine and orchid pollen to the cut stems of Coleus and Tradescantia. Callus formation and rooting were induced. Numerous other workers have since adopted lanolin as a diluent. The use of lanolin has persisted largely because of its desirable physical characteristics, and its apparent inertness toward plant tissues and growth substances.

An adaptation of the bio-assay method described by Laibach (1) was adopted in studies designed for the eventual isolation of the growth regulating substances in corn pollen. The degree of enrichment obtained from any particular fractionation was measured by the curvature produced on bean seedlings. By successive dilutions, determinations were made of the lowest concentrations dissolved in lanolin, which would produce curvatures of the same magnitude as would the non-diluted starting material. In earlier isolation attempts, even under the best processes, a considerable loss of activity sometimes occurred. It was found that upon standing for 24 hours at room temperature in contact with a 1% aqueous solution of hydrogen peroxide much of the growth regulator present in the crude pollen extracts was destroyed. Presumably, other oxidizing agents might also react with the growth regulator(s). Removal of peroxides from all solvents employed in extraction and enrichment processes did not eliminate the loss of activity indicated by the lanolin dilution assays. The probable source of this loss was detected when a qualitative test with acidified potassium iodide showed that the sample of lanolin employed as a diluent contained unidentified oxidizing agents.

Subsequently, it was found that treatment of the lanolin with sodium hydrosulphite removed the oxidizing agents. A product satisfactory for bioassay of natural growth substances may be obtained by shaking out a solution of 25 gm. of lanolin in 250 ml. of ether with 100 ml. of water in which are dissolved 15 gm. of sodium hydrosulphite and 5 ml. of glacial acetic acid. After separating the two phases, the less dense phase (ether)

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is washed with 100 ml. of water. The phases are again separated, and residual water is removed from the ether solution by agitating it with 15 gm. of anhydrous sodium sulphate, and the ether solution is evaporated to dryness over a steam bath. Fifteen ml. of water is then added and subsequently evaporated to dryness over a steam bath in vacuo. The residue from this second evaporation makes a satisfactory assay diluent.

Differences in the curvatures of bean seedlings which were observed when lanolin treated with sodium hydrosulphite was substituted for U.S.P. anhydrous lanolin are illustrated in Figure 1. Solutions containing 0.01, 0.001,

![Fig. 1](https://www.plantphysiol.org/)

Fig. 1. Curvatures induced on bean seedling internodes six hours after application of solutions of 3-indoleacetic acid in lanolin. Arrows point to sites of application. Left: Solutions in U.S.P. lanolin. Right: Solutions in purified U.S.P. lanolin. Top: 0.01% solutions of 3-indoleacetic acid. Center: 0.001% solutions of 3-indoleacetic acid. Bottom: 0.0001% solutions of 3-indoleacetic acid.

and 0.0001% indole-3-acetic acid were prepared both in U.S.P. anhydrous lanolin and in U.S.P. anhydrous lanolin which had been treated with sodium hydrosulphite. Five to ten mg. of each lanolin solution were applied unilaterally to the first internode of each of six bean² seedlings 72 hours after

² Variety *Tendergreen*, Ferry Morse Seed Co. Stock No. H6020.
emergence from the soil. Six hours after treatment three of the test plants from each group were photographed.

Distinct negative curvatures resulted from application of the 0.01% solutions of indole-3-acetic acid in both solvents (fig. 1). However, the growth substance in purified lanolin caused a more pronounced curvature than in non-treated lanolin. The application of a 0.001% solution in peroxide-free lanolin produced a marked response, while that in non-treated lanolin gave no perceptible curvature. A 0.0001% solution in either of the solvents produced no curvatures.

The use of U.S.P. anhydrous lanolin as a diluent for growth substances sensitive to peroxides may introduce an error of between 10- and 100-fold into quantitative assays. Caution must be observed in interpreting the results of assays in which the lanolin employed is assumed, without experimental justification, to be a completely inert solvent.

Summary

Lanolin has been found to contain sufficiently high concentrations of oxidizing agent(s) to interfere seriously with its use as a solvent for quantitative bio-assay of indole-3-acetic acid and for plant growth regulators in corn pollen. A method for removing the oxidizing agents from lanolin with sodium hydrosulphite is described.

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DEPARTMENTS OF AGRICULTURAL CHEMISTRY AND HORTICULTURE
MICHIGAN STATE COLLEGE
EAST LANSING, MICHIGAN

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