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DOSE-RESPONSE ANALYSIS OF SURFACTANT TOXICITY IN HYDROPONICALLY GROWN LETTUCE SEEDLINGS

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Catherine M. Greene

May, 1994

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ABSTRACT

DOSE-RESPONSE ANALYSIS OF SURFACTANT TOXICTY IN HYDROPONICALLY GROWN LETTUCE SEEDLINGS

by Catherine M. Greene

Igepon TC42, Ivory, and lecithin are three surfactants under consideration for use as hygiene soap in space habitats; the one chosen will be a major component of the habitats' gray water. Direct use of gray water in a hydroponic system is considered problematic because of the probable deleterious effects of surfactants on cell membranes. To quantify the phytotoxicity response to the candidate surfactants, a sensitive bioassay was utilized. Classic dose-response curves were developed for each surfactant. Of the three surfactants tested, Igepon and Ivory both exhibited acute toxicity thresholds of approximately 0.2 g L^{-1}. Lecithin was the least phytotoxic, exhibiting the highest toxicity threshold (0.8 g L^{-1}) and lowest unit toxicity. SEM analysis of primary roots exposed to phytotoxic levels of Igepon revealed epidermal shedding in extensive regions just proximal to the root tips. Cells in the cortex separated and formed perforations concomitant with abnormal lateral root development.

Chemical names used: N(coconut oil acyl)-N-methyltaurine (Igepon)

phosphatidylcholine (lecithin)

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INTRODUCTION

An increased effort to recycle human consumables has been made as the supply of raw material has become increasingly limited. It is especially critical during times of resource shortage, such as during drought, as well as in specialized environments, such as regenerative space habitat. The need to recycle and regenerate human consumables aboard long-term space missions is necessitated by the high cost ($22,000 kg^{-1}) of carrying mass in earth-to-orbit transport (Moses et al., 1989). Water contributes approximately 90% of the life-support consumables in a closed space environment (NASA SSP 30262, 1990). Reclaiming water from waste streams would help minimize the resupply costs associated with long-term space travel. Water reclamation can be accomplished by either physiochemical or biological processes. The NASA-sponsored program, Controlled Ecological Life Support Systems (CELSS), relies on plants to produce food and oxygen, to consume CO_2 by photosynthesis, and to produce potable water by transpiration.

It is estimated that, in a closed space station, an adult will utilize 29 L of water per day, about 45% of which is used for personal hygiene and dish washing (NASA SSP 30262, 1990). The primary contaminant of this used, "gray" water will be the cleansing agents, or soaps. The concentration of these soaps will be much greater than is typical of household sewage because of the water-conservation priorities. Igepon TC42 is the main ingredient of the soap currently recommended for showering and hand washing aboard Space Station Freedom. Ivory soap has been used in previous missions and remains a prospect for future missions. Lecithin is

added to many soaps as a skin softening agent and is a candidate for inclusion in a space-habitat soap formulation.

Recovery of gray water for potable and hygiene reuse is also essential in regions of acute or chronic water shortage, such as in heavily populated regions of the Southwest U.S., during drought, and in remote sites where water must be imported. Water supplies have been reduced as ground and surface water sources have become polluted or depleted. By the removal of soap components and other solutes in water by plants, and thus partial distillation and recycling, some of the strain on the limited domestic water supply would be alleviated.

Soaps have been applied directly to aerial plant surfaces in combination with insecticides, wetting and spreading agents, and emulsifiers for decades (Frear, 1942). Plants are tolerant of short periods of exposure at low concentrations, but we were concerned about long-term exposure of roots in hydroponic systems at high concentration. Surfactants are commonly used to disrupt plant cell membranes as a tool for gaining access to intracellular compartments. To investigate the use of waste water in hydroponic culture, we modified a sensitive bioassay (Berry, 1977) that utilizes seedlings of lettuce (*Lactuca sativa* L. cv. Waldmann's Green) to assess the phytotoxicity to Igepon, Ivory, or lecithin. By this procedure, the phytotoxicity threshold and unit toxicity of each of the three surfactants was quantified. Morphological changes to lettuce seedlings exposed to increasing Igepon concentrations were also characterized by scanning electron microscopy.

MATERIALS AND METHODS

Nutrient solution composition

Fifty lettuce seeds (*Lactuca sativa* cv. Waldmann's Green) were imbibed

in 1.0 L nutrient solution, formulated as follows and containing a soap. Macronutrients in the nutrient solution included 16.0 mM NO_3^-, 2.0 mM Mg^{2+}, 7.0 mM K^+, 5.0 mM Ca^{2+}, 2.0 mM SO_4^{2-}, and 1.0 mM HPO_4^{2-}. Micronutrients included 0.5 μM BO_3^{3-}, 9.0 μM Mn^{2+}, 0.7 μM Zn^{2+}, 0.3 μM Cu^{2+}, and 0.1 μM Mo^{2+}. Iron (17.0 μM) was added as a DTPA-sequestrene complex.

Surfactants

Anionic surfactants were added individually to nutrient solutions at the following concentration ranges: Igepon TC42 (Rhone-Poulenc, Cranberry, NJ) at 0-1.5 g L^{-1}, Ivory (Procter & Gamble, Cincinnati, OH) at 0-1.0 g L^{-1}, and lecithin ("Lecipur 95F," Lucas Meyer, Inc., Decatur, IL) at 0-2.0 g L^{-1}. Igepon TC42, N(coconut oil acyl)-N-methyltaurine, is a sodium salt of primarily saturated 12C (59.9%) and 14C (23.6%) fatty acids in which the carboxyl group is replaced by an amide to which is added a (sulfonated) taurine group to increase the detergent properties of the surfactant. Ivory is a sodium salt of a variety of carboxylated fatty acids, including C18:0 (29.8%), C12:0 (19.9%), C16:1(15.6%), and C16:0 (16.6%). Lecipur is composed of lecithin (phosphatidylcholine), a phosphoglyceride with variable fatty acid tails, chiefly including C16:0 (45%) and C18:1 (49%). Technically, a soap is a fatty acid processed by saponification; of the surfactants used in this study, Igepon and Ivory are soaps, whereas lecithin is unprocessed. For simplicity, we have called all of them soaps in this paper.

Bioassay

Seeds were germinated and the resultant seedlings remained in the solutions and were aerated and agitated continuously with filtered, compressed air. The solutions were maintained at 18°C and pH 7.0 under a

16 h photoperiod in a controlled temperature chamber with fluorescent and incandescent lamps, according to the specifications of Berry (1977). Five days after imbibition, primary root length was measured in ten seedlings from each treatment. Root length was plotted on a log-log scale as a function of surfactant concentration to generate dose-response curves for each of the soaps. The curves were characterized by the acute toxicity threshold (the lowest concentration of toxicant at which an additional dose causes a decrease in yield) and the unit toxicity (the decrease in yield per unit of toxicant after the threshold toxicity is surpassed) (Berry and Wallace, 1981).

Scanning Electron Microscopy

The roots of seedlings grown in Igepon for five days were fixed in 70% FAA (formalin-acetic acid-alcohol), dehydrated with tertiary butyl alcohol, dried in a Denton DCP-1 Critical Point Drier (Denton Vacuum, Inc., Cherry Hill, NJ, and sputter coated in a Hummer II (Technics, Inc., Alexandria, VA) with about 10 nm gold-palladium. Specimens were examined with a Cambridge Stereoscan Mark II SEM (Cambridge Instruments, a division of Leica, Deerfield, IL) and photographed on Polaroid 55 positive-negative film.

RESULTS AND DISCUSSION

Bioassay parameters

Conditions were determined for the lettuce seedling bioassay through a series of preliminary experiments. Lettuce seedlings grown in a pH series of 4.8-8.4 in nutrient solution showed no significant differences in primary root growth over a five-day period (Fig. 1). A midrange pH (7.0) was selected for the dose-response determinations.

A five-day period was chosen for the bioassay on the basis of a time-course study of root growth in nutrient solution (Fig. 2). A sigmoid growth pattern was realized over nearly a seven-day period, with five days being at the height of the linear growth phase. Although Savage et al. (1981) found that 2-4 lateral roots had formed in their lettuce seedlings grown under similar conditions, in our cultivar of lettuce, lateral root formation began shortly after five days. We wished to avoid the complication of additional organs and the possibility of compensatory growth between the primary and secondary roots and, therefore, chose an age in which lateral roots would be absent.

Dose-response

Lettuce seedling roots in the control group achieved a mean length of about 52 mm after five days in the nutrient solution. In the dose-response curves of all three soaps, slopes were near zero until the threshold concentrations were approached, indicating only a slight, if any, reduction in elongation of the roots at low soap concentrations (Fig. 3-5). Thus, below the threshold concentrations, mean primary root lengths were comparable to those of control seedlings. Acute toxicity thresholds to Igepon (Fig. 3) and Ivory (Fig. 4) were about 0.2 g L^{-1}, whereas lecithin (Fig. 5) produced a long tolerance plateau with a threshold about 0.8 g L^{-1}. Higher soap concentrations suppressed growth markedly, as reflected in the severe drop in the curves (Fig. 3-5). Reduction in root growth rate (steepest slope) at post-threshold concentrations was least in the lecithin (-11.0) and greatest in the Ivory (-41.2) solution (Fig. 4), indicating the lowest and highest unit toxicity, respectively, of the three soaps. Lecithin was least damaging to the plants at both low and high concentrations, most likely because it is a naturally occurring component of most biological membranes. Although the

mechanism of soap toxicity is unknown, the similar toxicity thresholds of Ivory and Igepon suggest that their mechanisms are similar, yet the unit toxicity of Ivory was more severe. The three-fold higher toxicity threshold of lecithin indicates that there may be more than a single mechanism of surfactant toxicity to lettuce roots.

Morphological phytotoxic responses

In control seedlings viewed under the SEM, root surfaces proximal to the cap were smooth and composed of regular, longitudinal files of epidermal cells (Fig. 6a). These specimens were similar to those grown in hydroponic solution and examined by SEM by Savage et al. (1981). The root cap surface was rough and irregular, as cells were being sloughed from the periphery (Fig. 6b), typical of healthy growing roots.

Seedlings exposed to Igepon concentrations (0.5 g L^{-1}) below the toxicity threshold showed limited cell damage in the 10-15 mm long region between the root cap and the root hairs (Fig. 6c). A few sloughed epidermal cells were visible as they clung to the surface, resembling control root caps (Fig. 6b).

In seedlings grown in Igepon and Ivory concentrations (1.5 g L^{-1}) above the toxicity threshold, browning began proximal to the root cap almost immediately upon emergence from the seed coat. The browned area expanded with root growth. In high concentrations of Igepon, epidermal tearing and shedding were evident in this region (Fig. 6d). Cellular debris with a filamentous, stringy appearance was produced on the surface, presumably as a result of shrinkage of the cellular debris after detachment from the root body and during SEM preparation (Fig. 6d). This region was well distal to the root hair zone, making negligible the probability that these filamentous structures were root hairs. In addition to the shedding of the

epidermis, numerous gaps could be seen in the underlying, newly exposed cortical tissue (Fig. 6d). Gaps that occurred along cell wall junctions appeared to have arisen by physical wall separation. However, many of the gaps were smaller than individual cells, were rounded in face view, and appeared as ruptures in the protoplasts, as might occur as a result of cell membrane damage (Fig. 6d). The resultant root mass was thinner and more fragile than that of the controls. The cotyledons, while showing no evidence of cell sloughing or other injury, remained smaller than those of the controls. Their smaller size may have been an indication of surfactant-induced damage to the root's absorptive function.

The browning of these five-day-old seedling roots was similar to that observed in 15-day-old lettuce roots after 4-6 hours in high concentrations (0.2 g L^{-1}) of Igepon by Wignarajah et al. (1992). Like those roots, the primary roots in our system did not die in the 5-7-day period for which they were observed. The damaged zone simply continued to expand, keeping pace with root elongation. It is, however, doubtful that root hairs would have been able to develop in the damaged region, and that alone might have led to eventual root abortion.

These phytotoxicity responses, root cell damage and reduced growth rates, are most easily explained by anionic surfactants interfering with cell surface membrane integrity. The amphipathic nature of the detergents allows them to insert themselves within a lipid bilayer to disrupt membrane structure. Similarly, root cell damage might be attributed to the chemical nature of fatty acids in solutions. Fatty acids form micelles that behave as detergents to disrupt protein and membrane structure (Voet and Voet, 1990). It is possible that micelle formation occurred in the phytotoxic Igepon

solutions, causing loss of cell membrane integrity leading to cell death and sloughing.

We considered the possibility that Igepon and Ivory, both sodium salts, acted through sodium toxicity. Heikal, et al. (1989) reported that sodium toxicity becomes evident among seedlings in solutions containing at least 8 mM Na^+. A 0.2 g L^{-1} Igepon solution contains 0.2-0.4 mM Na^+. These Na^+ levels are, therefore, too low to elicit the drop in the dose-response curve following the Igepon toxicity threshold of 0.2 g L^{-1}, as determined in this investigation.

Another possibility for the action of Igepon in seedling root growth inhibition is an osmotic mechanism to plasmolyze cells. However, even at the highest concentrations of Igepon used, the solute potential of the nutrient solution would be elevated only slightly, too little to account for the tissue tears and perforations observed.

There was little or no change in root growth over the pH range of 4.8-8.4 (Fig. 1), indicating that soap-related pH shifts within this range would not affect root growth. The pH of the hydroponic solutions used in this study remained within this range (data not shown). External pH changes within a range of 4-8 similarly had no effect on fresh weight of older seedling roots of lettuce, as determined by Arnon and Johnson (1942). Therefore, the soap-mediated reduction in root growth was not caused by a pH effect.

Seedlings grown in an Igepon concentration series developed lateral roots at different rates. Lateral root formation was evident 100 h after imbibition under Igepon concentrations of 0.4 and 0.6 g L^{-1} and 90 h after imbibition at 1.0 g L^{-1}. The browning and cellular rupture observed in the primary roots was mirrored in the secondary roots. In contrast, seedlings exposed to Igepon concentrations below the toxicity threshold did not

develop lateral roots during the five-day experiment. The primary roots presumably sensed the damage and compensated by initiating laterals to replace themselves, similar to increased lateral root formation under mildly toxic levels of copper, as reported by Savage et al. (1981). The mechanism did not apparently involve a reduction in root apical dominance as a result of apical meristem abortion, as the root tips maintained active growth in the presence of the soap. While the absorptive capacity of the primary roots was probably reduced by cellular rupture and shedding, there was no evidence of root hair impairment. In addition, seedlings grown hydroponically in the absence of nutrients achieved mean root growth rates comparable to, although with greater variability than, roots of seedlings grown in nutrient solutions. These roots were neither spindly nor fragile in appearance, as would be expected if roots were induced to elongate in response to low nutrient status. Therefore, it is unlikely that the Igepon reduced growth rates by nutrient binding or by causing cellular damage that resulted specifically in nutrient starvation.

Surfactant use

This investigation supports the use of plants within a CELSS to produce potable water by purification within their transpiration streams while producing food and replenishing atmospheric O_2. Seedling root growth was maintained at low concentrations of soap. Although growth was severely inhibited at higher concentrations, the seedlings did not die despite extensive root damage. Soap concentrations in gray water in space near or below the toxicity threshold would, therefore, support good plant growth in hydroponic solution.

In industrialized countries, the surfactant content in typical household sewage ranges from 0.005 to 0.025 g L^{-1}, generally averaging 0.010 g L^{-1}

(Swisher, 1987). The acute toxicity threshold concentrations determined in this study are well above these concentrations. Given chronic water shortage problems in California and other western states, the reuse of household gray water for watering yard and house plants appears to be a safe and effective means for recycling waste water; however, soil interactions with soaps must be assessed before the soap effects on these plants can be fully determined.

Although the acute toxicity threshold is well above household surfactant concentrations, water use in space is severely limited and higher surfactant concentrations are anticipated (Verostko et al., 1989). Total water usage in space for showering, personal hygiene, and housekeeping has been estimated at 5.44 L person^{-1} day^{-1} (Putnam, 1976). The total cleansing agent allotment per person per day in space is 1.4 g (Putnam, 1976), amounting to a total surfactant concentration of 0.26 g L^{-1}. This concentration is higher than the acute toxicity threshold for lettuce seedlings in Igepon and Ivory, but it would be reasonable to consider diluting gray water to maintain soap concentrations below 0.20 g L^{-1}. Only a 1.29 dilution factor would be needed to achieve the threshold concentration and so produce normal lettuce root growth. Alternatively, the soap allotment could be reduced. Growing plants is the only way to produce fresh food within a CELSS, and a reduction in yield would result if plants were grown in soap concentrations above 0.2 g L^{-1}. To maintain high productivity per unit area, more plants would have to be grown or gray water would have to be processed prior to plant exposure. Some accommodation for gray water phytotoxicity will be required in the design of a CELSS.

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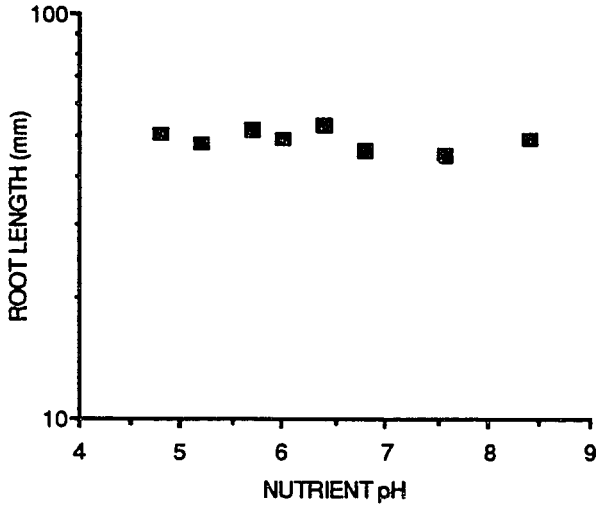


Figure 1. Growth of primary roots of lettuce seedlings for five days in nutrient solutions at 22°C and at different pH levels.

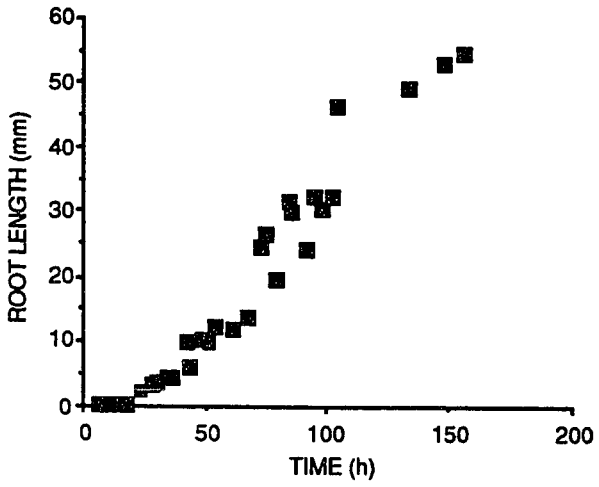


Figure 2. Growth of primary roots of lettuce seedlings in nutrient solutions at 20°C measured every eight hours for 155 hours. At the 120-hour point, growth began to taper, and lateral roots emerged.

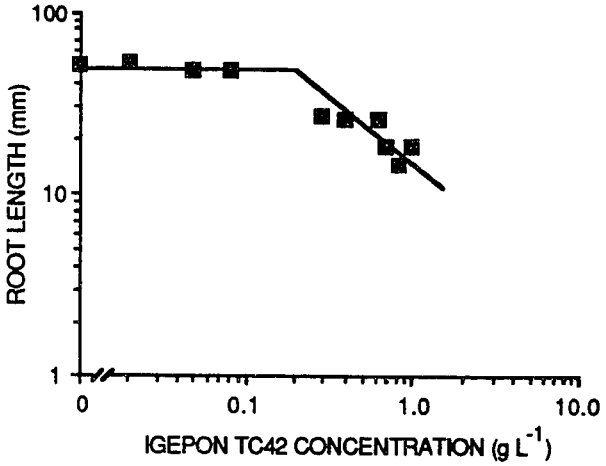


Figure 3. Dose-response curve of lettuce seedlings showing five-day effect on primary root length of a series of 22°C nutrient solutions, where the concentration of Igepon TC42 was increased from 0 to a toxic level (0-1.0 g L^{-1}).

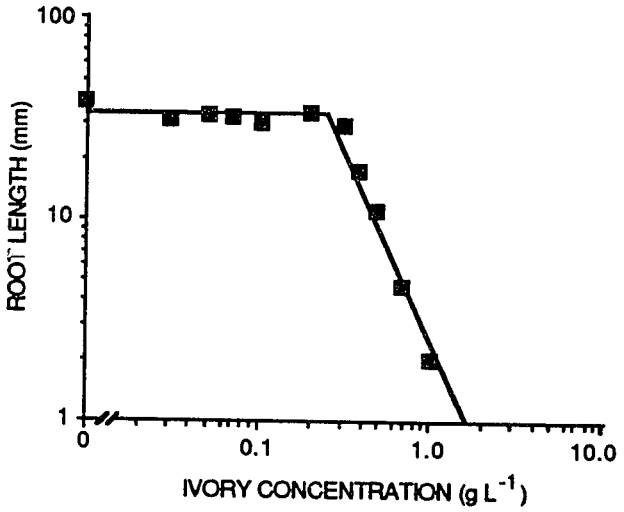


Figure 4. Dose-response curve of lettuce seedlings showing five-day effect on primary root length of a series of 19°C nutrient solutions, where the concentration of Ivory soap was increased from 0 to a toxic level (0-1.0 g L^{-1}).

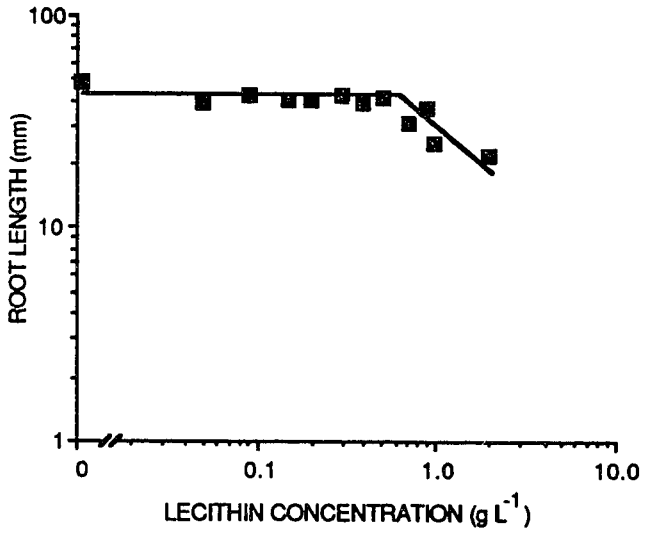
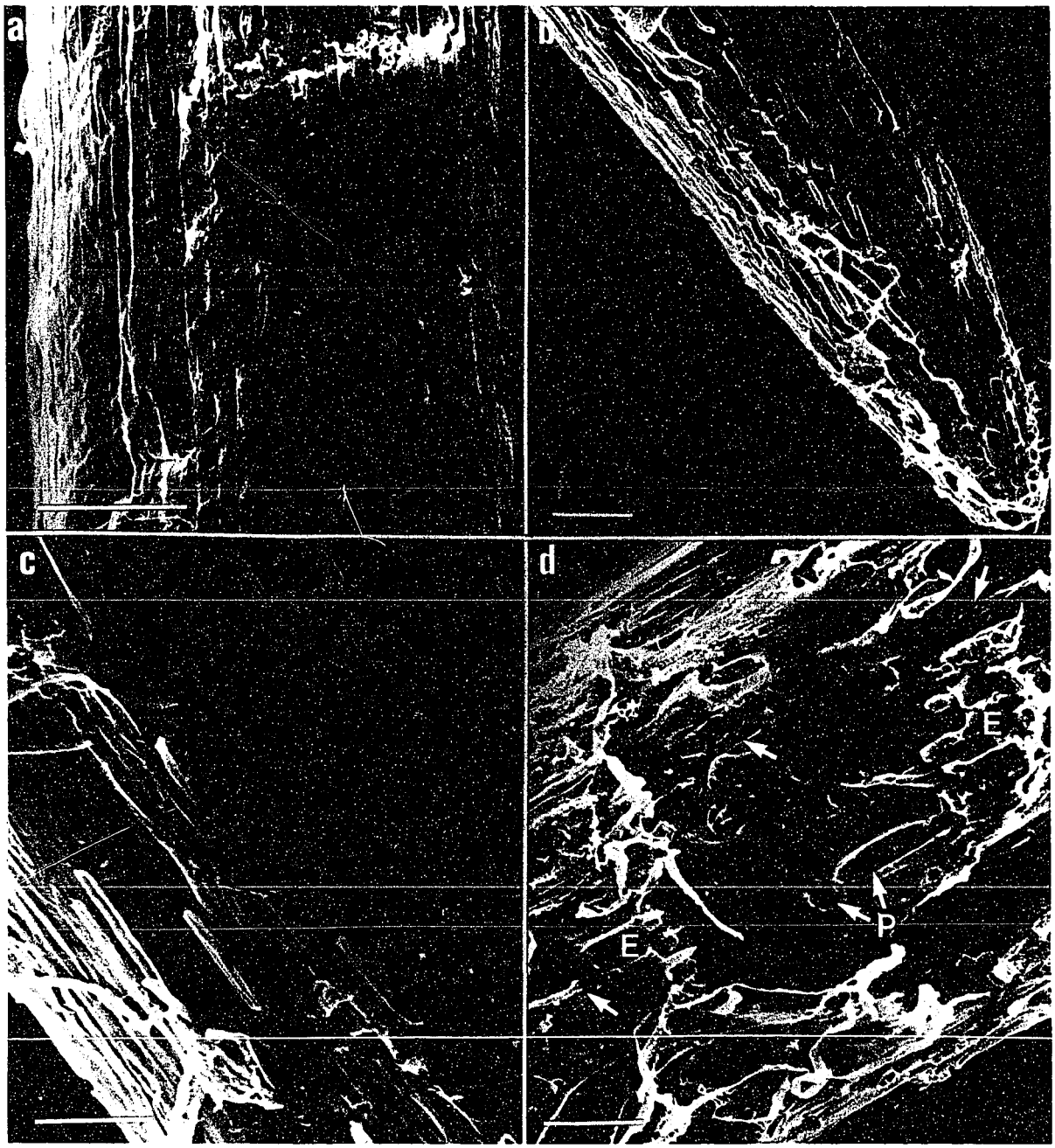


Figure 5. Dose-response curve of lettuce seedlings showing five-day effect on primary root length of a series of 21°C nutrient solutions, where the concentration of lecithin was increased from 0 to a toxic level (0-2.0 g L^{-1}).

Figure 6a-d. Scanning electron micrographs of primary roots of lettuce seedlings after five days in nutrient solutions. Bars = 0.1 mm. Fig. 6a. Nutrient solution lacking soap. Regular longitudinal files of turgid epidermal cells are evident without specimen preparation damage or damage from growth in hydroponic solution. X240. Fig. 6b. Nutrient solution lacking soap. Cell sloughing from the root cap. X130. Fig. 6c. Nutrient solution containing 0.05 g L^{-1} Igepon. Some shedding of individual epidermal cells, seen clinging to the root surface. X190. Fig. 6d. Nutrient solution containing 1.5 g L^{-1} Igepon. Increased epidermal cell shedding and other cellular injury evident in extensive region proximal to root cap and adjacent to remaining epidermal tissue (*E*). Cortical tears (at unlabeled arrows) arising from wall separation as well as perforations (*P*) presumably from cell rupture are also visible. X160.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