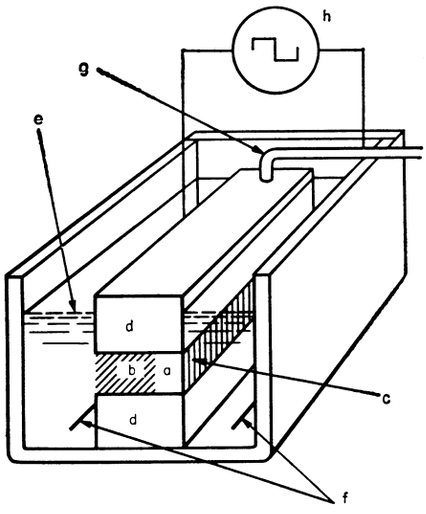


Fig. 1. Transverse alternating-current electrophoresis: *a*, separating column (1.5 by 1.5 by 240 mm); *b*, acrylamide gel reinforced with thick filter paper to give mechanical strength (1.5 by 50 by 240 mm); *c*, dialysis membrane; *d*, Plexiglas cooling plates; *e*, buffer level; *f*, electrodes; *g*, column inlet (or outlet); *h*, power supply giving a square wave output.

nents which were transported along the column by the buffer flow. In the reverse field the proteins migrated toward the dialysis membrane but they could not penetrate it. Since the forward and the reverse migrations of a protein may not be symmetrical, the timing cycle was adjusted to produce more migration toward the membrane than away from it, thus ensuring complete return of protein from the gel

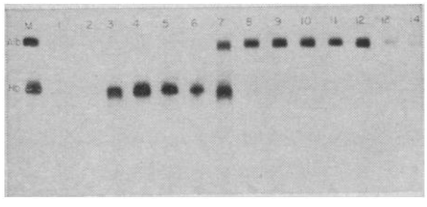

Fig. 2. A gel electropherogram showing the composition of the fractions (22 $\mu$l) from the electrophoretic column described in the text. For each cycle the column was operated at 300 volts for 85 seconds into the gel, and then for 95 seconds out of the gel. The buffer was a tris-ethylene-diaminetetraacetate-borate formulation (*4*) diluted to an ionic strength of 0.01. The gel was 7 percent Cyanogum-41 prepared in the same *p*H 8.4 buffer. The flow rate along the column was 33 $\mu$l per minute. After fraction 8, the voltage was reduced to 200 volts. Two hundred microliters of a mixture (*M*) containing 4 percent human carboxyhemoglobin (*Hb*) and a complex of 4 percent bovine serum albumin and bromphenol blue complex was initially injected into the system.

to the buffer stream. The extra migration time toward the dialysis membrane ensured complete mixing (transversely) within the buffer stream through gravitational convection and electrodecantation. Such transverse mixing is an essential step in the operating cycle (*2*). The entire cycle was repeated many times as the protein sample migrated down the column.

To begin the operation, the electric field was applied and reversed periodically, and the buffer was allowed to flow until the column reached a steady state. A small protein sample dissolved in buffer was then injected into the inlet of the column. Voltage and timing of the cycle were adjusted so that approximately one-half of the protein migrated into the gel at each reverse half-cycle, and all of it returned to the buffer stream at the forward half-cycle. In our prototype column, the sample size and the flow-rate were adjusted to permit approximately six cycles to operate before the leading protein component began to emerge from the column.

The mathematical analysis of conditions within the column, taking into account hydrodynamic flow, thermal convection, electrodecantation, and other effects, is more complex than can be conveniently presented here, but the following conclusions can be experimentally substantiated. (i) With a constant electrophoretic cycle, components emerge from the column in reverse order of their electrophoretic mobilities. (ii) By increasing the voltage, a protein of finite mobility can be retained in the column for as long as desired. (iii) Gradient elution can be achieved by initiating operation with a higher voltage and then reducing voltage gradually to elute components with higher mobilities (in sequence).

For a preliminary demonstration of these principles, a mixture of human hemoglobin and a complex of serum albumin and bromphenol blue was used so that the process could be followed visually (Fig. 2). The recovered fractions were analyzed by conventional gel electrophoresis (*1*). Despite the large sample volume employed (in comparison to channel volume), which limited the number of times the field reversal could be applied, the two components were completely separated.

This apparatus also offers interesting possibilities in the study of electrophoretic processes. Extremely high fields can be applied for very short periods, without exceeding the average power input capacity of the column, making it possible to study potential effects of high voltages in electrophoretic transport (*3*).

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2. Without transverse mixing at this point in the operating cycle, reversal of migration direction would merely restore the separation obtained in the first cycle. It is important, therefore, to obtain transverse mixing while minimizing longitudinal (that is, in the direction of flow) mixing which degrades the separation achieved.
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20 June 1966

## Chemical Pruning of Plants

Abstract. *The lower alkyl esters of the $C_8$ to $C_{12}$ fatty acids and the $C_8$ to $C_{10}$ fatty alcohols selectively kill or inhibit the terminal meristem without damaging the axillary meristems, foliage, or stem tissue of a wide variety of plants. The axillary meristems develop at nearly the same time as those of plants whose terminal meristems have been removed manually. The concentration of the fatty acid esters and alcohols required to kill the terminal meristem range from 0.025 to 0.05*M *for herbaceous plants, 0.05 to 0.16*M *for semi-woody plants, and 0.16 to 0.27*M *for woody plants.*

Tso (*1*) reported that the alkyl esters of the $C_9$ to $C_{12}$ fatty acids inhibited the growth of axillary buds when applied to tobacco plants whose tops had been removed. Only the meristematic cells of the buds were destroyed by the chemical. The leaves of the treated plants were unaffected. The methyl ester was found by Tso, Steffens, and Engelhaupt (*2*) to be more effective than the isopropyl and butyl esters.

We report that lower alkyl esters of the $C_8$ to $C_{12}$ fatty acids and the $C_8$ to $C_{10}$ fatty alcohols in aqueous emulsions selectively killed the terminal meristems of a wide variety of plants without damaging the axillary meristems, foliage, or stem tissues of the plants. The plants were sprayed with the minimum amount of emulsion to wet all surfaces. The first visible

response occurred within 15 minutes after plants of certain species had been sprayed with an emulsion of the fatty acid esters or alcohols. The terminal meristems initially turned black, and became flaccid within 30 to 60 minutes. The tissue turned tan-colored within 3 to 5 days. The axillary meristems developed at nearly the same time as those of plants whose terminal meristems had been removed. (The meristems were routinely removed manually.) The first internodes on the axillary shoots of plants treated with the fatty acid esters and alcohols tended to be longer than those on plants whose terminal meristems had been removed. The number of axillary shoots developing may be greater and at a wider angle to the main stem than on plants with the terminal meristem removed (Fig. 1). Some plants treated with more than optimum amounts of the fatty acid esters or alcohols developed axillary shoots 3 to 15 nodes from the apex of the stem. Thus fatty acid esters and alcohols in excess may kill both the terminal and axillary buds of the plant without damaging foliage and stem tissue.

Action of the fatty acid esters and fatty alcohols depends on use of the proper surfactants. Fatty acid esters and alcohols are insoluble in water. Responses of the plants to emulsions prepared with high-frequency sound with no added surfactants are very irregular; some leaves are injured without killing the terminal meristem (Fig. 1). Surfactants with a high hydrophile-lipophile balance (HLB) are more effective surfactants than those with a low HLB. Use of certain surfactants results in emulsions which lack stability, cause plant injury, or do not allow for sufficient penetration. In our formulations the fatty acid methyl ester and fatty alcohols were blended with equal weights (or less) of surfactant. Sufficient water was added to form a gel. Water was then gradually increased to form a colloidal emulsion which was opalescent and stable.

Equivalent amounts of certain fatty alcohols are more active than the corresponding fatty acid methyl esters on some plant species, and less active on others. Their effective non-phytotoxic range is narrower than that exhibited by the fatty acid esters. Inclusion of 0.26*M* dimethylsulfoxide (DMSO) enhanced the kill by the emulsions of fatty acid esters and alcohols on woody plants. An emulsion of the fatty acid ester or alcohol made with less than the optimum amount of surfactant caused foliar necrosis.

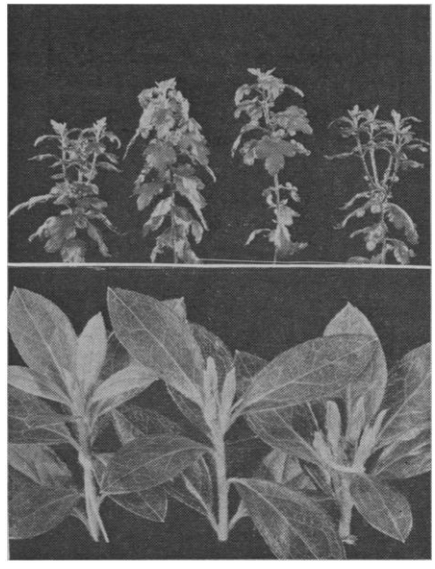

Fig. 1. (Top) Plants of "Improved Indianapolis Yellow" chrysanthemum grown at 17°C and treated as follows. Left to right: plant with terminal meristem removed; untreated plant; plant sprayed with an insonated emulsion of 0.16*M* methyl decanoate; plant sprayed with a colloidal emulsion of 0.16*M* methyl decanoate and 1 percent equal weights of Tween 21 and Span 20 (*8*). (Bottom) Shoots of 'Red Wing' azalea grown at 17°C and treated as follows. Left to right: untreated plant; plant with terminal meristem removed; plant sprayed with a colloidal emulsion of 0.16*M* methyl nonanoate, 0.26*M* dimethylsulfoxide (DMSO), and 1 percent equal weights of Tween 21 and Span 20. Photographs made 21 and 27 days after treatment.

Of the materials we have tested thus far, methyl nonanoate and methyl decanoate cause chemical pruning at the following concentrations: herbaceous plants (ageratum, coleus, cotton, marigold, peanut, snapbean, snapdragon, soybean, tomato), 0.025 to 0.05*M*; semi-woody plants (carnation, chrysanthemum, forsythia, geranium, hydrangea, poinsettia), 0.05 to 0.16*M*; and woody plants (apple, azalea, chamaecyparis, elm, euonymus, juniper, kolkwitzia, ligustrum, lonicera, maple, paper birch, pyracantha, taxus, weigela, pear), 0.16 to 0.27*M*.

The stage of growth determined the sensitivity of the plants to the chemicals. Plants with resting terminal meristems exhibited no response to concentrations of 0.16 to 0.27*M* methyl decanoate. Death of active terminal and axillary meristems often follows treatment with the chemicals. The sensitivities of the two meristems were very similar. Marigold and snapbean, for example, exhibited chemical pruning when sprayed with 0.05*M* emulsions of methyl decanoate. Concentrations higher than 0.05*M* killed all meristems, and in this case no regrowth of the axillary buds occurred. *Coleus* exhibited the same initial response as marigolds and snapbeans, but eventually secondary axillary meristems grew. The terminal meristems of plants of barley and red fescue were completely enclosed by the sheaths of older leaves. The chemicals damaged the leaves at concentrations greater than 0.05*M* without affecting the terminal meristems.

The action of fatty acids and alcohols differs from that of other chemicals in that it selectively destroys or inhibits the terminal meristem without inducing undesirable effects, as other chemicals often do. For example, maleic hydrazide (*3*) inhibits growth of axillary meristems in tobacco but induces further metabolic changes which are considered undesirable to leaf quality. Also *N*-(1-naphthyl)-phthalamic acid and related compounds (*4*) inhibit terminal growth and accelerate growth of axillary shoots of beans, but are inactive on many other plants. Two compounds, 3-chloroisopropyl-*N*-phenylcarbamate and the ammonium salt of 3-chloro-methoxyphenylacetic acid, reduce axillary shoot growth of tobacco but also cause a slight epinasty of the petioles (*5*). Triiodobenzoic acid is physiologically active in altering the growth habits of soybeans but it is relatively inactive on many plant species (*6*). Finally, in algae fatty acids act as uncouplers in photophosphorylation (*7*).

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### References and Notes

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8. Mention of a trade name does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture nor an endorsement by the Department over other products not mentioned.

24 June 1966