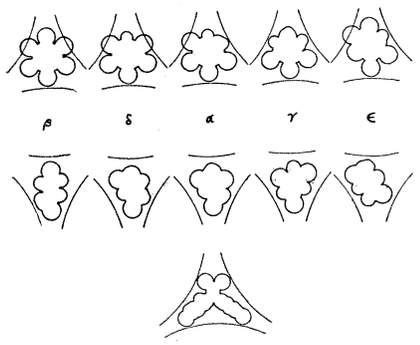

Fig. 1. Scale drawings from Stuart models showing the fit of various BHC isomers into a membrane interspace for plane (top) and one end-on (middle) orientation. The interspace (shown by curved lines) is formed by three cylindrical membrane molecules with a diameter of 40 A at a separation from each other of 2 A. All plane orientations, except that of γ-BHC, are excluded, although end-on orientations of all the isomers are permitted. At the bottom the only possible orientation of DDT is shown.

attracted to only one of the three surrounding membrane molecules, thus forming an interspace block but not a distortion.

If the foregoing model is to have any generality, it ought to be capable of accounting for the action of DDT and its various analogs. Diphenyltrichloroethane is capable of occupying the interspace in several orientations, one of which is with one benzene ring parallel to the surface, and another is with both benzene rings in an end-on orientation. The former orientation is not one in which the attractive forces of the halogen atoms can best be made effective, whereas the latter makes use of the attractive forces of not only the halogens but also the two benzene rings. To direct the orientation of diphenyltrichloroethane to end-on, *p,p'*, and, to a lesser extent, *m,m'* substitution with some group of the approximate size of chlorine is effective. If the *p,p'* substituent is made too large (for example, iodo or propoxy), penetration will be delayed or inhibited. By increasing the distance across the molecule (from the chlorine of trichloroethane to *p*-Cl), *p,p'* substitution makes it impossible for DDT to penetrate other than in an end-on orientation, as is shown at the bottom of Fig. 1.

Penetration in this orientation depends very much upon having the two benzene rings in an approximately end-on orientation. The free rotation of the benzene rings that is possible in *p,p'*-dichlordiphenylethane is constrained by 1,1,1-trichloro substitution in the ethane nucleus (*6*). This orientation of the two rings can be disturbed by a number of changes in the DDT molecule, such as by chlorination of the 2 position in ethane and by *ortho* chlorination of either of the benzene rings, changes that tend to rotate the benzene rings with respect to each other. These compounds are all much less active than DDT. Subtraction of halogen from the ethane nucleus is also capable of disorienting the rings, although this becomes important only when two or three halogen atoms have been removed. The change from DDT to dichlorodiphenyldichloroethylene increases markedly the distance between the chlorine atoms of dichloroethylene and their corresponding benzene rings (because of the change in bond angles), and hence makes the molecule nonpenetrating. The dichloroethylene compound is also relatively inactive. The steric situation responsible for the toxicity of DDT would thus appear to involve two factors: (i) a specific orientation of the two benzene rings with respect to each other, and (ii) a distance along the two axes of the molecule (ethane Cl to *p*-Cl) great enough to prevent a plane orientation of one of the rings in the interspace.

L. J. MULLINS

Biophysical Laboratory,
Purdue University, Lafayette, Indiana

References and Notes

1. This work was aided by a grant (B-139) from the National Institute for Neurological Diseases.
2. L. J. Mullins, *Chem. Rev.* 54, 289 (1954).
3. J. Ferguson, *Proc. Roy Soc. (London)* B127, 387 (1939).
4. J. H. Hildebrand and R. L. Scott, *The Solubility of Non-Electrolytes* (New York, 1950).
5. Space does not permit the derivation of this interspace size from experimental data on narcosis. In a more extensive paper it will be shown that the interspace size need not be set arbitrarily.
6. A somewhat contrary view is taken by R. Riemschneider [*Z. Naturforsch.* 9, 95 (1954)] who considers DDT analogs as most effective when free rotation of the benzene rings is possible. What has really been noted here is that ring substitution that tends to disturb the normal orientation of DDT results in less active compounds. This does not account for the usually diminished activity in dichlorodiphenylethane where free rotation is really possible.

25 April 1955

Improved Automatic Microtome

The automatic microtome I described in *Science* [**115**, 649 (1952)] that cuts and mounts serial sections of imbedded biological specimens on a 35mm strip of film base has been made more effective by two changes in the manner of its use: (i) specimens are mounted in tissuemat, and (ii) Mylar film is used so that it is possible to stain the sections after cutting.

Originally the instrument cut doubly imbedded specimens. It is preferable to use tissuemat, because it is routinely employed by many laboratories and because it saves imbedding time. The cutting of tissuemat sections works well under all conditions encountered, and they adhere well to the Mylar film without the use of an adhesive coating when the automatic microtome is carefully adjusted.

Staining previously was done on the tissue *in toto,* largely because of the damage suffered by acetate film in the various solvents. With Du Pont's Mylar film, which is not affected by solvents, we are now able to remove the tissuemat and stain the sections on the film in standard solutions.

Freshly cut sections are coated with a thin celloidin solution 0.5 percent and a thin coat of lacquer. Eastman film lacquer has been found to be excellent for the purpose. This treatment secures the sections to the film so that they do not become displaced in subsequent processing. A strip of 5 ft, or about 80 serial sections, can be wound on the reel of a 35mm developing tank such as the Nikor tank. The tissuemat is then extracted with Xylene, and the regular process is continued, using a series of solutions of alcohol. When the dyeing is completed, and the strip is again in Xylene, it is removed from the tank, and a second piece of Mylar film is placed on top of the strip, using a mounting medium. Longer strips can be used with automatic photographic developing equipment.

Projection or examination under the microscope is facilitated, since ordinary 35mm film-handling equipment can be used to bring successive sections into register. Copies may readily be made with ordinary motion-picture equipment.

VANNEVAR BUSH

Carnegie Institution of Washington, Washington, D.C.

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Elemental Sulfur Dust, a Nutrient for Lemon Leaves

Certain citrus growers in California have observed that they were able to pack more first-grade fruit from citrus trees that had been dusted with elemental sulfur dust than from nondusted trees. Losses due to injured peels of fruit unprotected from the sun's direct rays were to be expected, but these were considered negligible when they were compared with the increase in first-grade fruit obtained. It is not known how sulfur dust improved the grade of fruit or what unobserved physiological effects sulfur had. It became apparent to one of us, however, while he was assisting in the preparation of "Standard Values in Nutrition and Metabolism" (*1*), that elemental sulfur applied to foliage of higher plants has not been demonstrated to penetrate the foliage and to enter into the anabolic processes therein.

Vaporization of elemental sulfur has been reported (*2*), and vapors from elemental S^{35} dust have been shown to penetrate the peel of lemon fruit (*3*). S^{35} dust, as well as H_2S^{35}, $S^{35}O_2$, and $H_2S^{35}O_4$, has been shown to penetrate the peel of lemons and to produce S^{35}-labeled proteins (*4*). Fried (*5*) has shown, also, that $S^{35}O_2$ gas enters the inorganic and the soluble and insoluble organic portions of the plant by absorption in aerial parts. The present study (*6*) was undertaken because it thus seemed possible that elemental sulfur dust used as an insecticidal and/or fungicidal agent might, under some conditions, in other ways benefit the plants to which it was applied.

Sets of undusted leaves on adjoining twigs of "Y" branches (Fig. 1) on the shady side of a Eureka lemon tree were covered with cellophane bags that had a single pinhole opening on the underside to permit the drainage of condensed moisture. These bags were tied tightly to the branches to prevent any sulfur dust from reaching them from the leaves on twigs of the other part of the "Y" which were then dusted on their upper and lower surfaces with elemental S^{35} dust, using a camel's-hair brush. The dusted leaves were then covered with cellophane bags, which were also tied tightly to prevent loss and spread of the S^{35} dust (Fig. 1). Leaves on another portion of the tree, not on "Y" branches, were also bagged before S^{35} dusting was done to serve as controls.

Certain pairs of nondusted leaves on the "Y" branches with S^{35}-dusted leaves were selected during the period between date of treatment, on 18 Aug. and 23 Sept. for determination of the radioactivity in the soluble-sulfur fraction. The remainder of the leaves in the experiment, which were harvested on 12 Oct. 1954, were thoroughly scrubbed with soap and water and a soft brush, rinsed in distilled water, and dried between cotton towels. The leaves were then macerated in a Waring Blendor with 200 ml of water, and the proteins were isolated

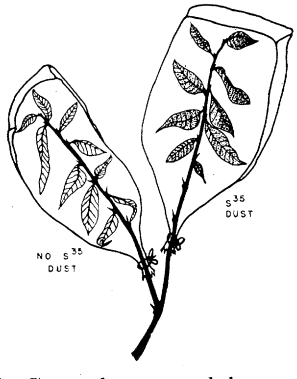

Fig. 1. Covered untreated lemon leaves and covered S^{35}-dusted leaves on a "Y" branch.

Table 1. Specific activity of sulfur and protein constituents of S^{35}-dusted and nondusted lemon leaves

Constituent	Protein in fresh leaf (%)	Sulfur in protein, in ash (%)	Specific activity of protein (counts/sec mg)	Specific activity of $BaSO_4$ (counts/sec mg)
		S^{35}-dusted "Y" leaves		
Acid-soluble protein	3.771	0.0017	0.162	9.909
Alkali-soluble protein	0.480	1.4903	2.847	1.678
		Nondusted "Y" leaves		
Acid-soluble protein	2.553	0.2780	0.000	2.107
Alkali-soluble protein	0.094	0.9358	0.007	0.331
Water extract		0.1096		0.823
		Nondusted leaves		
Total ash		0.1449		0.025

by the method of Sinclair *et al.* (*7*). The sulfur in the dry protein and in the water-soluble materials was isolated as $BaSO_4$ by standard methods (*8*), was plated out at less than 2 mg/cm² on aluminum planchets, was weighed on a microbalance to 1 μg, and was counted in a flow counter. A portion of the isolated proteins was collected on filter paper, and the S-impregnated paper was counted. The counting data have been fully corrected to absolute values.

It can be seen in Table 1 that both the acid-soluble and the alkali-soluble proteins of the leaves dusted with S^{35}-labeled elemental sulfur dust became radioactive and showed a high specific activity in the $BaS^{35}O_4$ obtained from them. The elemental sulfur not only penetrated the leaf but also entered the metabolic stream and was synthesized into protein. There is a remote possibility, however, that the sulfur dust was not entirely removed from the leaf in the scrubbing or that traces of radioactive $H_2S^{35}O_4$ known to be produced by the action of the atmosphere on elemental sulfur (*9*) may have penetrated the leaf in the washing process.

On the other hand, the presence of S^{35} in the nondusted leaves on the branches of the "Y" that were covered with cellophane bags prior to the sulfur dusting of the leaves on the other side of the "Y" would not be open to the foregoing criticisms. If such nondusted leaves contained S^{35} compounds, although no S^{35} came in contact with their surfaces, then such S^{35} as they contained would have had to be transported from leaves dusted with S^{35}; and if the proteins of such leaves contained radioactive sulfur, the S^{35} must have been transported from the treated leaves while both sets of leaves were on the tree and before synthesis into protein could take place.

The results of analyses show that the specific activity of the sulfur in the protein of these leaves was very low or negligible; but the $BaSO_4$ obtained from the protein ash showed a significant specific activity (Table 1). The low specific activity of the isolated protein probably resulted from absorption of the weak β-particles by the filter paper on which it was counted. But since the $BaSO_4$ from the protein was radioactive, it may be concluded that elemental sulfur may act as a source of sulfur for the nutrition of higher plants.

Water extracts of nondusted leaves on the "Y" branch contained sulfur precipitable as $BaSO_4$ having more than 10 times the specific activity of the $BaSO_4$ from water extracts of the whole ash of leaves on this tree but far removed from the site of sulfur-treated leaves. The relatively high specific activity of the $BaSO_4$ from water extracts of leaves on the nondusted sides of the "Y" suggests that sulfur is transported from the site of the sulfur-dust to other locations in the plant as inorganic SO_4 and as such is available for nutrition of the tree.

F. M. Turrell
J. R. Weber
Department of Plant Biochemistry, University of California Citrus Experiment Station, Riverside

References and Notes

1. E. C. Albritton, *Standard Values in Nutrition and Metabolism* (American Institute of Biological Science and National Research Council, McGregor & Werner, Dayton, Ohio, 1953), Table 9, p. 19.
2. F. M. Turrell, *Science* 105, 434 (1947).
3. ———, *Plant Physiol.* 25, 13 (1950).
4. ——— and M. B. Chervenak, *Advances in Chem. Ser.* 1, 250 (1950).
5. M. Fried, *Soil Sci. Soc. Amer. Proc.* 13, 135 (1948).
6. This work was financed in part by the U.S. Atomic Energy Commission under Contract AT(11-1)34, Project 6. Sulfur^{35} was obtained from the U.S. Atomic Energy Commission, Oak Ridge, Tennessee. Paper No. 850, University of California Citrus Experiment Station, Riverside, California.
7. W. B. Sinclair, E. T. Bartholomew, R. D. Nedvidek, *J. Agr. Research* 50, 173 (1935).
8. H. A. Leeper, *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists* (Assoc. of Official Agricultural Chemists, Washington, D.C., 1945).
9. W. H. MacIntire, F. J. Gray, W. M. Shaw, *Soil Sci.* 11, 249 (1921); J. W. Mellor, *A Comprehensive Treatise on Inorganic and Theoretical Chemistry* (Longmans, Green, New York, 1930), vol. X, pp. 88, 91–92.

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