# SCIENTIFIC APPARATUS AND LABORATORY METHODS

#### APPARATUS FOR DUSTING SULFUR ON PLANTS IN CONTROLLED AMOUNTS

WHILE making comparative studies on different brands of sulfur, it became necessary to secure dusting apparatus that would deliver quantitatively small amounts of sulfur to the under surface of leaves. Since many brands of sulfur stick tenaciously to dusting equipment and at the same time produce little or no fog, the common methods of applying dust under laboratory conditions were not satisfactory. The apparatus herein described has been satisfactory for the purpose intended and may be of value to other workers.

A dust gun was made from a small glass cylinder 80 mm by 15 mm (A) fitted with two corks. Into one cork was inserted a metal tube with a 1 mm opening which admitted compressed air. The air was under 20 pounds pressure and controlled by a valve (B) obtained from a cheap spray gun. From the other cork led a glass tube fire polished to a 1 mm opening. This glass tube connected by means of a rubber tube (C) with the dusting chamber. The dusting chamber con-



sisted of a large wooden box enclosing a phonograph turn table (D) which was used to transmit power to a cookie can (E) held at a  $45^{\circ}$  angle with the open end down. Plants grown in flower pots were inserted into the cookie can and revolved as the phonograph

turned. The sulfur came into the dusting chamber, hit a glass plate (F) and diffused evenly over the revolving plant. When filling the glass dust gun, a small rod was held in the center of the gun and was removed later. This left a small cylinder of dust, which was gradually removed in its entirety by the force of air passing through the center of the sulfur cylinder on its way to the dusting chamber.

The dust gun may be adapted into a very useful small hand duster (G) by attaching a rubber bulb to the glass tube and by placing a cheese cloth over the other end. A rod is held in the center of the glass cylinder while filling in the same manner as described above. The cheese-cloth serves as a screen to prevent coarse particles from leaving the duster and at the same time diffuses any large puffs of dust that might be emitted.

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#### A NEW STAINING METHOD FOR STRUC-TURES OF THE SPINAL CORD

DISADVANTAGES in staining of cytons and Nissl bodies (chromophilic bodies) of the spinal cord may be overcome by employing dyes that contain greater amounts of methylene violet. Polychromed methylene blue does not contain sufficient methylene violet to prevent fading, especially in combination with acidic contrast dyes, such as eosin. The Nissl methylene blue stain is polychromed with castile soap and allowed to age for some time before use, but fading occurs in a short time if an acidic counterstain is used. Cytons become destained within one week.

By employing the Giemsa stain and differentiating in 95 per cent. and absolute alcohol, a brilliant effect was obtained. However, fading within a few days was noticed when an acidic counterstain was employed.

In order to obtain the advantages of selectivity and permanence the following mixture of dyes and timing was arrived at:

Five parts of a solution of Wright's blood stain in 95 per cent. denatured ethyl alcohol to one part of a standard solution of Giemsa was prepared. The spinal cord of a steer, fixed in 10 per cent. acid-free formaldehyde, was sectioned at 10 mu. Slides were passed through xylol and graded alcohols to distilled water and flooded with the above mixture of dyes for two minutes. The dye was then diluted with an equal amount of distilled water for two minutes and the slides then immersed in fresh distilled water for one minute. The sections were passed immediately into 80 per cent. alcohol for 15 seconds and the dehydration rapidly completed in 95 per cent. and absolute. Sections were cleared in neutral xylol and mounted in neutral balsam.

Dehydration must be rapid so that cyton areas do not become completely destained. After washing in 80 per cent. alcohol the sections will become reddish in color with blue cyton areas. If the dehydration is properly carried out, the following structures will be selectively and permanently stained:

Cytons and Nissl granules deep blue; nuclei of blood vessel structures and neuroglia light blue; elastic fibers of blood vessels deep blue; erythrocytes pink; and neuroglia fibers light red.

The intensity of the cyton stain may be increased or decreased by varying the proportion of the Giemsa solution in the mixture.

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### A RAPID METHOD FOR REMOVING COVER GLASSES OF MICROSCOPE SLIDES

IT is often necessary in cytological work to remove the cover glass of a slide, in order to replace a broken cover glass or restain the sections underneath. For this purpose most workers use xylene. The writer, however, has found that a mixture of 90 parts of xylene and 10 parts of n-butyl alcohol acts much more rapidly. The hard and brittle balsam or damar of old slides, which would require an immersion of several days in pure xylene, is usually dissolved by this mixture in a few hours. This time difference is probably due to the presence-especially in old slides -of a small amount of moisture in the mounting medium around the margin of the cover glass. Such moisture would offer a barrier to the penetration of pure xylene, but not to xylene containing n-butyl alcohol; for the latter is miscible with small amounts of water, as well as with xylene, balsam and damar. It should be remembered that butyl alcohol is a solvent of the aniline dyes, and so material stained with these substances will be destained in this xylenebutyl alcohol mixture.

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# SPECIAL ARTICLES

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## X-RAY DIFFRACTIONS FROM HEMOGLOBIN AND OTHER CRYSTALLINE PROTEINS

SEVERAL attempts have been made during the past years to get x-ray diffraction photographs of the crystalline proteins. Most of these<sup>1</sup> have not been successful, but in a few instances very simple patterns have been observed.<sup>2</sup> These patterns, which always consisted of two rather broad and diffuse rings, have been found from proteins as different as edestin, excelsin, egg albumin and hemoglobin.

The diffuseness of the rings, combined with their simplicity irrespective of diffracting substance, suggests the pattern of a glass or other amorphous material rather than of a crystal. The probability that they are such amorphous patterns is strengthened by the recent statement<sup>3</sup> that a typical sharp line pattern can be prepared from a single crystal of pepsin left in its mother liquor.

We have been seeking to obtain truly crystalline powder patterns from edestin, excelsin and hemoglobin. Photographs prepared in the usual way from (1) commercial edestin, (2) well-crystallized edestin and excelsin freshly made from hemp seeds and Brazil nuts and (3) crystalline (white rat) oxy- and carbon

monoxy-hemoglobin gave the familiar "amorphous" bands. When these preparations were examined microscopically they proved to be more or less completely altered after photography. Further microscopic study demonstrated that the protein crystals always decomposed rapidly on exposure to air. From the way this disintegration took place it was clear that they all contained water of crystallization which was very readily lost.

Photographs with copper K radiation were accordingly made of the wet crystals sealed into thin containers having windows of 0.01 mm glass. Under such conditions the protein crystals remain unchanged and typically crystalline patterns, consisting of fine, though very faint, lines, are produced. With this experimental arrangement reflections corresponding to large spacings lie too close to the central image for accurate measurement. Additional, and far more instructive, photographs have consequently been made with the longer chromium K radiation by keeping samples in moist chambers without protective windows. Some spacings thus measured on typical pictures of rat oxyhemoglobin are listed in Table I. There is no reason to believe that we have yet established the largest spacings that exist for this hemoglobin or for the other protein crystals. Our techniques, however, are being improved and it is expected that these maximum spacings will eventually be determined.

<sup>&</sup>lt;sup>1</sup> For example, R. O. Herzog and W. Jancke, Naturwiss., 9: 320, 1921; W. H. George, Proc. Leeds Phil. Soc., 1: 412, 1929.

<sup>&</sup>lt;sup>2</sup> See J. R. Katz, "Die Rühtgenspeldungungehie als Untersuchungsmethode" (Berlin, 1931), p. 188. <sup>3</sup> J. D. Bernal and D. Chukfurd, Nature, 133: 794,

<sup>1934.</sup>