process into another active principle, possibly acidione itself. The important point is that the chemical structure of these derivatives of actidione is such as to enable them to be taken up readily through the leaves and translocated to protect new growth against infection for a period of at least 3 weeks. Data at hand suggest that the practical implication of these findings on systemic control is the minimization of the importance of timing of sprays and the reduction in the number of applications now required for protection.

Further studies on the behavior of the derivatives of actidione systemically and otherwise may well provide some helpful keys in unfolding the mysteries of the mechanism of chemical disease control in plants.

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New Method for Mass Determinations on Microscopic Particles

The determination of the dry mass of microscopic particles is of value in histochemical and cytochemical studies dealing with the growth and development of cells and tissues. At the present time, interference methods are generally used for this type of investigation (*1*).

This report deals with a new method of estimating quantitatively the dry mass within single cells. The principal difference between the previously described methods and the method described here is that this new method is based not on optical interference but on an optical schlieren system. It eliminates, however, the disadvantage of some schlieren equipment, particularly those used in electrophoresis instruments, which render no true image of the object.

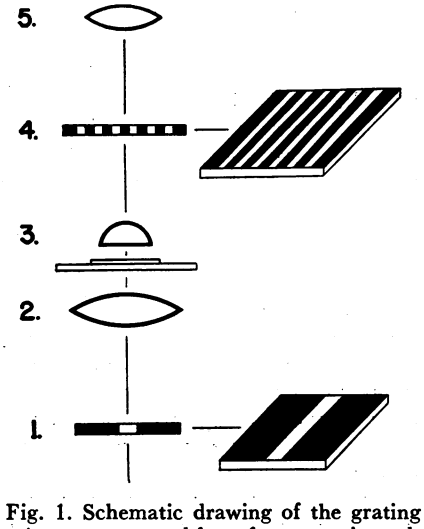

Fig. 1. Schematic drawing of the grating microscope as used for refractometric studies under higher magnification.

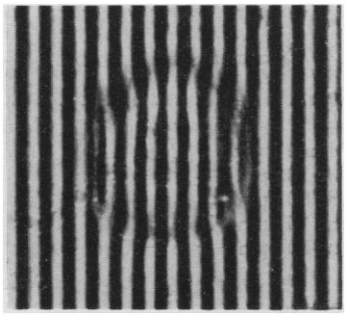

Fig. 2. Photomicrograph of the refractometric pattern of a living yeast cell, obtained by means of the grating microscope. (× 600.)

Our method has the additional advantage that no specially constructed objectives and cover slips are necessary and that no special preparation techniques have to be employed. Only an optical absorption grating and a slit diaphragm, for work at low magnifications, must be fitted to any standard microscope. The usual low- and high-power objectives can be used. In contrast to interference methods, the distance between the lines depends not on the wavelength of the light but only on the fineness of the grating. Nevertheless, in order to obtain sharper lines that are free from color dispersion effects, the use of monochromatic light is recommended.

The optical principle utilized is shown in Fig. 1. A standard microscope is equipped with a slit diaphragm (1) inserted below the condensor and movable around the optical axis. The slit lies in the lower focal plane of the condenser (2). Objectives with a magnifying power from 2.5 to 100 times can be used (3). A grating (4), bearing 175 lines per inch, is placed approximately at one-half the distance between the objective and the eyepiece (5) in the tube of the microscope.

Usually, the slit of the diaphragm and the lines of the grating are arranged to be parallel to each other. If they are not parallel, or if the slit diaphragm is removed, the lines of the grating are not discernible in the microscopic field. In this condition, the object can be examined by the usual microscopic technique, and an area for refractometry can be selected. Then, if the slit is turned to a position parallel to that of the grating lines, the lines become distinct and form the background, as can be seen in Fig. 2. This is owing to the fact that the slit now acts as a pointlike light source projecting the grating as a shadow into the viewing lens.

Figure 2 shows the refractometric pattern of a living yeast cell immersed in water. The contour of the cell can easily be recognized by the typical displacement of some lines, especially those that are located most laterally. Under the assumption that the object is small and that its upper and lower surfaces are flattened by the pressure of the cover slip, the amount of anhydrous protein can be computed by using the formula

$$C \sim n_M \cdot \frac{D}{p} \cdot \frac{1}{\alpha}$$

C represents the number of grams of anhydrous protein per 100 ml of cytoplasm; n_M is the refractive index of the mounting medium; D represents the displacement of a line in relation to its former position; p is the distance of the displaced part of the line from the geometric center, measured in the plane of the grating; α is the specific refractive increment, for which a factor of 0.0019 is generally accepted for proteins (*2*).

In the case of the yeast cell, shown by Fig. 2, a value of 42 g/100 ml of cytoplasm for the dry mass (C) was computed. This is in close agreement with values obtained by interference microscopy. Although this schlieren method is less sensitive than interferometric refractometry, the necessary equipment is much less expensive and easier and more convenient to operate, and we recommend this method for practical mass determinations in cytochemistry.

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References and Notes

1. S. Tolansky, *Multiple Beam Interferometry of Surfaces and Films* (Oxford Univ. Press, London, 1948); A. M. Frederikse, *Z. wiss. Mikroskop.* 52 (1935); H. G. Davies *et al.*, *Quart. J. Microscop. Sci.* 95, 271 (1954); J. Dyson, *Nature* 171, 743 (1953); R. C. Mellors, A. Kupfer, A. Hollender, *Cancer* 6, 372 (1953).
2. An account of the derivation of this formula is in preparation.

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Evidence for the Incorporation of Iron and Calcium into the Pigments of Serratia marcescens

Waring and Werkman (*1*), in their study of the iron requirements of certain bacteria, demonstrated that iron was necessary for both the growth and pigmentation of *Serratia marcescens*. This effect had been noted earlier by Bortels (*2*) who makes the further statement