browning as reported by Shiroya and Hattori (*6*). Thioglycollic acid inhibited browning with no loss of chlorogenic acid. Thiourea inhibited browning with a loss of chlorogenic acid and the appearance of a new spot; this suggests a function other than chelation of copper (*7, 8*).

Extracts of cabbage were also tested. After standing for 1 hour, the extract did not brown, but as is shown in Fig. 1, a new, yellow fluorescent spot ($R_f = 0.08$) appeared on the chromatogram from the nonboiled cabbage extract. This spot gave an immediate reddish-brown color after it had been sprayed with ammoniacal silver nitrate, and it gave a faint test for sulfur with the KI-H_2PtCl_6 reagent. A definite ninhydrin test was obscured by the abundance of free amino acids in the extract. Chlorogenic acid and two other bluish-white fluorescent areas were evident in both the boiled and nonboiled cabbage extracts.

Figure 2 shows the ultraviolet absorption spectra of alcohol solutions of the cysteine-, glutathione- and cabbage-chlorogenic acid compounds obtained after extracting their corresponding yellow fluorescent areas from paper chromatograms.

In view of the widespread occurrence of *o*-phenolic substrates and polyphenoloxidase in plant tissues, the possible *in vivo* operation of these reactions requires further study.

ROBERT E. HENZE
Department of Biochemistry, Purdue University, Lafayette, Indiana

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13 February 1956

Systemic Control of Cherry Leaf-Spot Fungus by Foliar Sprays of Actidione Derivatives

In an evaluation of the antibiotic actidione (cycloheximide) on potted Montmorency cherry trees in the greenhouse, it was found that this material is highly specific in fungicidal activity against the cherry leaf-spot fungus, *Coccomyces hiemalis* Higgins, not only as a protectant, but also as an eradicant of the fungus after it is established in the leaf [J. M. Hamilton and M. Szkolnik, *Phytopathology* **43**, 109 (1953)]. The testing of certain derivatives of actidione for their eradicative activity during the 1954 season led to the discovery that the oxime derivative is effective systemically in the control of the cherry leaf-spot fungus.

Table 1. Control of cherry leaf spot systemically with the oxime derivative of actidione.

Treatment	Interval between spraying and inoculation	Leaf spot on new leaves: Lesions per 2 in.²*
	Series A, 26/July/54	
Actidione 1 ppm	2 weeks	83 ±
Oxime 10 ppm	2 weeks	20
Oxime 60 ppm	2 weeks	Trace
Oxime 10 ppm + lime 2–100	2 weeks	42
Oxime 60 ppm + lime 2–100	2 weeks	19
	Series B, 11/Apr./55	
Unsprayed	1 week	49
Actidione 1 ppm	1 week	50
Oxime 30 ppm	1 week	17
Oxime 60 ppm	1 week	4
	Series C, 12/May/55	
Unsprayed	3 weeks	39
Oxime 30 ppm	3 weeks	20
Oxime 60 ppm	3 weeks	4
	Series D, 27/Oct./55†	
Unsprayed	5 days	69
Oxime 10 ppm	5 days	39
Oxime 60 ppm	5 days	13
Semicarbazone 10 ppm	5 days	2
Isomer 10 ppm	5 days	1
Acetate 10 ppm	5 days	5
Anhydro 10 ppm	5 days	17
Dehydro 10 ppm	5 days	32

* Average of four heaviest infected new leaves per shoot.
† Averaged for the two to three new leaves per shoot. The derivatives of series D were prepared by the Upjohn Co., Kalamazoo, Mich.

Greenhouse-grown cherry trees were sprayed with the test materials, kept in the greenhouse for the development of new growth, and inoculated with *C. hiemalis* 2 to 3 weeks later. Following inoculation, the trees were placed in a saturated humidity chamber for 48 hours at 18.5°C to provide conditions suitable for infection by the fungus. The trees were then returned to the greenhouse for completion of the incubation period.

It was found that the oxime at 60 ppm was taken up by sprayed foliage and translocated to new growth in sufficient amounts to provide protection against infection by the leaf-spot fungus (Table 1). Actidione does not exhibit this systemic activity. The systemic protection by the oxime extended through the seven to nine new leaves that grew during the 2- to 3-week interval between initial spraying and subsequent inoculation. There was a significant reduction of shoot growth with the oxime derivative at concentrations greater than 60 ppm, but injury is not considered a serious factor at the 60-ppm level. The oxime derivative lost part of its stability in the presence of hydrated spray lime.

The oxime derivative at 40 ppm was effective in inhibiting further development of the cedar-apple rust, *Gymnosporangium juniperi-virginianae* Schw., in the foliage of potted Rome Beauty trees when it was applied 24 hours after inoculation or when the lesions were just becoming visible.

Inasmuch as the semicarbazone derivative of actidione also gave indication of being systemically active in early tests, other derivatives of actidione were evaluated in 1955. The isomer, acetate, and semicarbazone derivatives were found to be definitely systemic at 10 ppm or lower concentrations, which are within the economical level for agricultural use. The anhydro derivative also exhibited systemic activity but not as much as that of the three derivatives just mentioned. Injury with these derivatives at 10 ppm or lesser concentrations was of no significance in the greenhouse tests but ranged from moderate to severe at 60 ppm. Among the factors to be explored more fully are the interrelationship of concentration, the number of leaves sprayed for chemical uptake, and the extent of new growth to be protected.

The isomer, acetate, and semicarbazone derivatives were protective against the leaf-spot fungus at 0.5 ppm and gave good eradicative control at 1 ppm when they were applied 4 days after inoculation.

It has not yet been determined whether these derivatives remain active as such within the plant tissue or whether they are altered through some biochemical

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.

J. M. HAMILTON
MICHAEL SZKOLNIK
ERNEST SONDHEIMER
Departments of Plant Pathology and Food Science and Technology, New York State Agricultural Experiment Station, Cornell University, Geneva

20 February 1956

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.

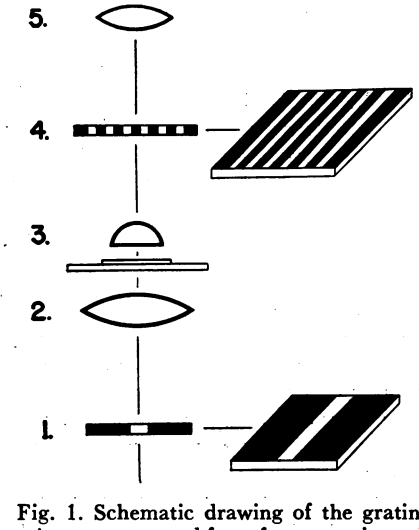

Fig. 1. Schematic drawing of the grating microscope as used for refractometric studies under higher magnification.

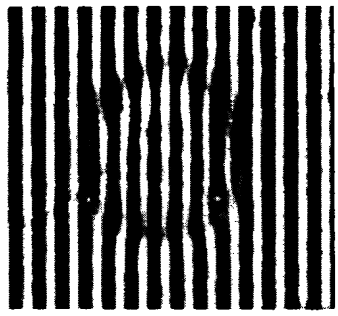

Fig. 2. Photomicrograph of the refractometric pattern of a living yeast cell, obtained by means of the grating microscope. (× 600.)

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.

JURGEN MEYER-ARENDT
Department of Pathology, Ohio State University, Columbus

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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