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.

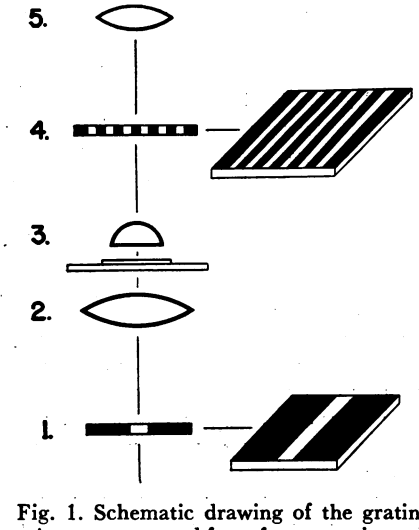

Fig. 1. Schematic drawing of the grating microscope as used for refractometric studies under higher magnification.

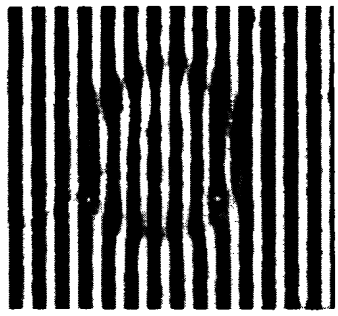

Fig. 2. Photomicrograph of the refractometric pattern of a living yeast cell, obtained by means of the grating microscope. (× 600.)

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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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

Table 1. Distribution of Fe^{59} in *S. marcescens* cells and pigments. Total Fe^{59} counts in 70 ml of sample was 3.4×10^6 count/min.

Fraction	Weight (mg)	Total count/min in fraction	Specific activity (count/min mg)
Dry cells	750	1.2×10^6	1.6×10^3
Entire pigment	1.00	10.4×10^3	10.4×10^3
Blue component	0.51	8.3×10^3	16.3×10^3
Combined red components	0.49	1.3×10^3	2.7×10^3

Table 2. Distribution of Ca^{45} in *S. marcescens* cells and pigments. Total Ca^{45} counts in 70 ml of sample was 11.9×10^6 count/min.

Fraction	Weight (mg)	Total count/min in fraction	Specific activity (count/min mg)
Dry cells	760	2.8×10^6	3.7×10^3
Entire pigment	1.00	1.2×10^3	1.2×10^3
Blue component	0.50	0.65×10^3	1.3×10^3
Combined red components	0.50	0.51×10^3	1.0×10^3

that he could find no evidence for the presence of iron in the pigment itself. Bortels stated that Samkow (*3*) had found iron to be a constant component of the ash produced after combustion of the pigment.

During the course of chemical investigations carried out on the pigment components, which had been separated by paper chromatography (*4*), we found that the pigment fractions contain a large amount of ash. Most of the ash could be removed by treatment with disodium ethylenediaminetetraacetate (Versene). A qualitative thiocyanate test established that the ash contained iron. Spectrochemical analysis (*5*) demonstrated that other metal contaminates also were present in the ash, the principal ones being, in addition to iron, calcium and magnesium. We have investigated the presence of iron and calcium in the pigment fractions by use of the radioactive isotopes of the metals (*6*).

Serratia marcescens, strain Nima, was grown in liter flasks containing a thin layer of a liquid casein hydrolyzate-yeast extract medium to which isotopic Fe^{59} or Ca^{45} had been added. The cultures were incubated for 7 days at 30°C, and then the cells were harvested. The pigment was extracted and fractionated into its components by paper chromatography according to methods described elsewhere (*4*). The amount of isotopic iron in the various components was determined by means of a scintillation counter; the amount of isotopic calcium, by means of a thin-window Geiger-Müller tube.

The results of the experiments carried out with Fe^{59} and Ca^{45} are presented in Tables 1 and 2. Owing to the fact that the various red components (*4*) were present in quantities too small for accurate isotopic counts to be made, the three red fractions were combined to form the "combined red components." Examination of the tables reveals that both iron and calcium are incorporated into the cellular components of *S. marcescens*. However, the uptake of iron is greater. Approximately 34 percent of the added isotopic iron is found in the cells, whereas only 24 percent of the isotopic calcium was incorporated. Distribution of the isotopes in the various fractions according to their specific activity demonstrates that iron is specifically incorporated into the pigment. Calcium, however, is present to a lesser degree in the pigment than in the intact cells. The specific-activity calculations also emphasize the fact that iron is incorporated into the blue fraction of the pigment about 6 times as much as the metal is incorporated into the combined red fractions. Calcium is distributed more or less equally between both pigment fractions.

These results establish the fact that *S. marcescens* pigments will incorporate metals. However, the exact interpretation of the data must await the elucidation of the chemical structure of the pigment fractions. It is interesting to note that Wrede and Rothhaas (*7*) stated that the pigment was a tripyrrole compound. This structure would provide three nitrogen atoms as potential electron donors whose electrons could be shared with polyvalent metals in a chelate association. The increased affinity of the pigments for iron in lieu of calcium could be explained by the fact that iron can exist as a trivalent ion, but calcium is always divalent. Spectral and chemical data indicate that the blue component is a possible dimer of the red components (*8*). Such a structure could explain the greater binding of iron in the blue fraction, since more combining sites would be available than in the red fractions of the pigment.

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Lengthening the Carbon Chain of Sugars

Sugars that have more carbon atoms than the starting compounds have been prepared by several methods involving the addition of hydrocyanic acid, nitromethane, diazomethane, and the Grignard reagent to the appropriate sugar. A critical evaluation of these methods has been presented by Pigman and Goepp (*1*).

We have now found (*2*) that the carbon chain of carbohydrates can be lengthened by the addition of carbon monoxide and hydrogen to unsaturated sugars (glycals, *1, 3*). To the best of our knowledge, this is the first application of the oxo reaction (*4*) to the field of carbohydrates.

To a solution of 8.5 g of 3,4,6-tri-O-acetyl-D-galactal (*5*), 5.8 ml of ethyl orthoformate, and 0.4 g of dicobalt octacarbonyl (*6, 7*) in 20 ml of purified benzene contained by the liner of a reaction vessel having a void of 280 ml, there was added carbon monoxide (200 lb/in.^{2}) followed by hydrogen (1670 lb/in.^{2}) After the reactants had been heated at 130° to 140°C for 3 hours (pressure drop of 170 lb/in.^{2} at 22°C), the inorganic material was removed, and evaporation under vacuum yielded 9.3 g of a sirup. The products were chromatographically fractionated (*8*) using acid-washed aluminum oxide as absorbent and benzene-ethanol as developer. Two fractions were obtained. The lower (A) fraction (yield, 8.0 g) was deacetylated using 0.05*N* sodium methylate, and the product was then deionized using Amberlite 1R-120. The product was recrystallized several times from a solution of methyl alcohol in ethyl acetate (paper chromatograms showed one zone). Determined specifications of the product were as follows: mp = 158.5° to 159.5°C