amounts, is found above the isolutein on the adsorption column. The former has been found in all the leaves examined (those of barley, carrots, filaree, miner's lettuce, sunflower, squash and spinach) and is identical with zeaxanthin isolated from the calyx of *Physalis alkekengi* with respect to melting point, optical activity, quantitative absorption spectrum of its solutions, absorption maxima in different solvents, adsorption upon Tswett columns and elementary composition. The zeaxanthin is followed upon the column by a pigment, the absorption curves of which are similar to those of flavoxanthin (absorption maxima 4510 and 4220 Ångström units in ethanol). The pigment is optically active ($[\alpha]^{18}_{6678}=+75°$ in chloroform). Another similar pigment, which exhibits a like absorption curve (absorption maxima 4510 and 4220 Ångström units in ethanol) but opposite optical rotation ($[\alpha]^{18}_{6678}=-56°$ in chloroform), is adsorbed next on the column. It is followed by considerable quantities of a xanthophyll, the absorption curves of which are intermediate in shape between those of lutein and those of the flavoxanthin-like pigments (absorption maxima 4667 and 4374 Ångström units in ethanol). Upon partition between 60 per cent. methanol and petroleum ether, this pigment was distributed almost entirely in the methanol. It is followed on the column by several xanthophylls, which occur in small quantities and which remain adsorbed near the top of the column, even after the latter has been washed repeatedly with fresh solvent.

It now seems established that several of the adsorbed pigments observed by Tswett are mixtures of two or more xanthophylls. Many of these are not identical with previously described substances of this nature and, even though many of them occur in small quantities, together they constitute a considerable proportion of leaf xanthophyll.

The physical and chemical properties of the leaf xanthophylls are being redetermined. When this is accomplished, a complete description of the compounds isolated and of the methods used in their preparation will be published elsewhere.

The observations recorded in this note are of particular significance in relation to methods for the quantitative estimation of leaf xanthophyll. The variations in the composition of leaf xanthophyll, which result from different methods of preparation, and the great differences in the absorption spectra of the different constituents preclude the use of most xanthophyll preparations as standards or reference substances for the determination of xanthophyll concentration by comparative spectral absorption methods. The heterogeneity of leaf xanthophyll and the variations in properties of the constituents account, no doubt, for the divergent values which have been reported for the xanthophyll content of leaves.

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THE AVERAGE PORE SIZE OF DIAPHRAGMS[1]

IN connection with some investigations of the relationship between surface charge density of adsorbed ions and ionic antagonism,[2] it became necessary to evaluate the mean pore radius of cellulose and powdered quartz diaphragms.

The expression for electrical conductivity,

$$C=\frac{l}{\pi r^2 n},$$

(where C is the cell constant and n the number of capillaries of mean length, l, and mean radius, r) was substituted into Poiseuille's law,

$$V=\frac{\pi P r^4 n}{8 \eta l}.$$

Here V represents the rate of flow in cc per sec. under a pressure, P, in dynes per cm.[2] The resultant equation,

$$r=\sqrt{\frac{8 \eta C V}{P}},$$

can be used to estimate r, assuming a capillary of circular cross-section (as, in general, with most of such methods) and a membrane of non-conducting material. A relationship of this degree of simplicity does not appear to have been previously described.

Diaphragms were made by packing the desired material into a streaming potential cell of the type used by Martin and Gortner.[3] The origin of the quartz and cellulose used has been described before.[4] The cell constant was measured with the diaphragm filled with N/10 KCl to eliminate the effect of surface conductance. Since the pierced gold electrodes form the ends of the cell, electrical conductance and liquid flow data can be directly compared. Any narrowing of the effective pore radius by imbibition of water should be accounted for by this method.

The rate of flow was found to be linear with pressure and independent of time; no blocking effects could be noted. The radius of the quartz diaphragms investigated ranged from 1.4 to 30 μ, depending upon the degree of grinding. On the other hand, cellulose membranes were more uniform in behavior, with a

[1] Paper No. 1355 Journal Series, Minnesota Agricultural Experiment Station.

[2] L. S. Moyer and H. B. Bull, *Jour. Gen. Physiol.*, 19: 239, 1935.

[3] W. M. Martin and R. A. Gortner, *Jour. of Phys. Chem.*, 34: 1504, 1930.

[4] H. B. Bull and R. A. Gortner, *Jour. of Phys. Chem.*, 35: 309, 1931; 36: 111, 1932.

radius of 0.88–0.98 μ. The mean radius of cellulose diaphragms was found to be independent of the electrolyte concentration, whereas the values for finely ground quartz rose with increasing salt concentration to a constant value at 4×10^{-4} N NaCl. These data illustrate the use of this simple method, which should prove of value in other investigations of this character. A more detailed discussion of these results has been presented in a later paper.[5]

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE APPARATUS TO FACILITATE THE PREPARATION OF BACTERIAL VACCINES[1]

ONE of the main problems in preparing bacterial vaccines on a large scale is to prevent contaminations during the process of preparation. Since a large number of bottles are used in preparing vaccines and each bottle must be opened several times, it is frequently difficult to avoid contamination. The usual procedure in collecting the bacterial growth from the inoculated bottles is to add saline to each bottle and collect the bacterial suspension into a sterile bottle by means of volumetric pipettes. This is of necessity a slow procedure and the vaccine is exposed to contaminations repeatedly; because the collecting bottle must be opened a number of times to discharge the contents of the pipette.

A simple apparatus has been devised which eliminates the use of pipettes and reduces the risk of contaminations to a minimum. The apparatus consists of a special burette for adding saline to the bottles containing the bacterial growth, as illustrated in Fig. 1, and a tube for collecting the vaccine, as shown in Fig. 2. The burette is connected serially by means of rubber tubing to a bottle of sterile saline, an empty sterile bottle and a bottle of 5 per cent. phenol. These bottles have two-hole rubber stoppers, glass tubes and rubber-tube connections (see illustrations, Fig. 1). The burette, collecting tube and the connections are sterilized separately and are assembled aseptically before use. All parts of the apparatus are protected against contamination. The top of the burette is plugged with cotton, which is wrapped in cheese-cloth and after plugging is covered with a paper cap. The delivery point of the burette is protected by a glass bell, which is also covered with a paper cap. The open end of the rubber tube on its side arm is placed in a glass tube, which is plugged with cheese-cloth. Similarly, all open parts of the connections for the bottles (Fig. 1) and the collecting tube (Fig. 2) are protected by glass tubes. They are then wrapped in paper separately and are sterilized in the autoclave for one hour at 15 pounds pressure. After the apparatus is assembled, as shown in Figure 1, the bottle containing the phenol is connected with a pressure pump. The metal clamps "h" are opened and air is forced through the phenol which sterilizes it before it reaches the saline. The intermediate empty bottle serves as a safety valve in case too much pressure should force the carbolic into the adjoining bottle. The burette is filled by opening cock "a" on the side-arm, which is closed when the saline reaches the zero mark. The desired amount of saline is then added to the bottles containing the bacterial growth by opening and closing cock "b" on the delivery tube. Both the mouth of the bottle and the delivery point of the burette are protected by the glass bell. During the entire operation all parts of the apparatus are completely protected against contamination.

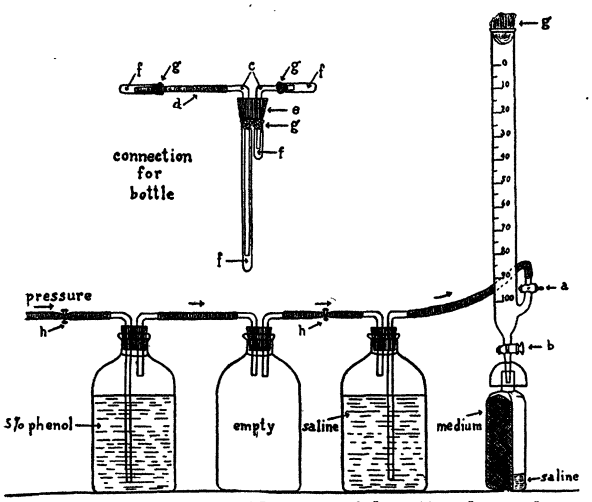

FIG. 1. a—cock or side arm of burette; b—cock or delivering tube of burette; c—glass tubes of connection; d—rubber tube; e—two-hole rubber stopper; f—glass tubes; g—plugs; h—metal clamp.

When the saline is added to all the bottles the burette is replaced by a sterile collecting tube, as illustrated in Fig. 2. This consists of a glass tube that has a constricted and slightly bent point. This tube is protected from contamination by an outer glass tube whose diameter is the same size as the neck of the bottle containing the bacterial suspension. The collecting tube is held in place by a perforated rubber cap and it slides back and forth in the outer tube. The outer tube serves a two-fold purpose—it protects the collect-

[1] From the Bureau of Laboratories, New York City Department of Health; Director, W. H. Park, M.D.

[5] H. B. Bull and L. S. Moyer, *Jour. Phys. Chem.*, 40: 9, 1936.