a quick and more even heating of the three walls of the moist chamber. Although slight, the expansion of the water within the inner chamber and the escape of small gas bubbles, which are very gradually formed, into the overflow tube probably aid also in this regard. Controlled temperatures ranging from a room temperature of 18° C to 48° C can be obtained within the moist chamber. The variation from a given temperature is, at the most, about ± .3° C.

The thermostat is operated by the expansion and shrinkage of air in the sealed activating chamber against the column of mercury in which the adjustable platinum wire electrode dips. The dimensions of the activating chamber, the connecting side arm, and the rear arm of the U-tube are such that the mercury column is moved approximately 1 mm for each °C change in temperature. When the air in the activating chamber expands, it simply pushes the meniscus of the mercury column toward, and finally away from, the end of the adjustable electrode. This breaks the electrical circuit and interrupts the heating device. When the air in the activating chamber shrinks due to cooling, the mercury column rises into contact with the tip of the adjustable electrode to complete the circuit and to activate the heating device. The thermostat is set by lowering the adjustable electrode to increase the temperature and by raising it to decrease the temperature.

This instrument is accommodated by any standard microscope and is used with the Chambers micromanipulation assembly in all respects just as if it were a standard moist chamber slide (*2*, *4*).

Detailed measurements necessary for the construction of the constant-temperature micromanipulation chamber may be obtained by applying the 1″ scale in Fig. 3 to the construction plans (Figs. 2A, B, C; Fig. 3). The mercury

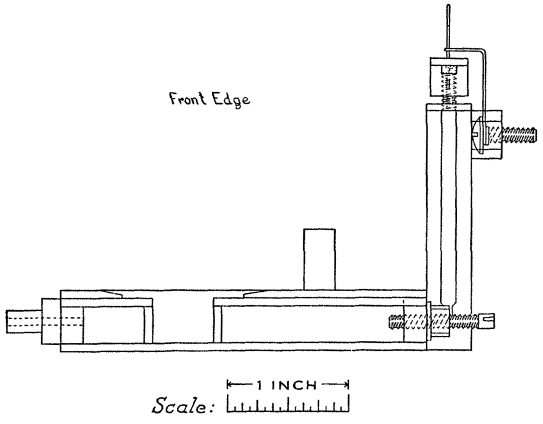

FIG. 3

is placed in the modified U-tube in two steps: (1) With the seal plate (Fig. 1X) off, mercury is added to the U-tube to the general height shown in the front arm of the U-tube in Fig. 1. (2) The seal plate (Fig. 1X) is welded into position, additional mercury is added to the rear arm of the U-tube to a point about 10 mm below the junction of the side arm, and this arm of the U-tube is then sealed with the appropriate assembly (Fig. 1).

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The Use of Thionyl Chloride in the Preparation of Schiff's Reagent

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While studies on the specificity of the Feulgen reaction were being conducted, methods for the preparation of Schiff's reagent were evaluated. Since sulfur dioxide is the agent active in the conversion of basic fuchsin to its leuco form, it does not appear to matter what source for this compound is used so long as secondary products are not produced which could affect the reaction between the dye and the sulfurous acid. Sulfite, bisulfite, and metabisulfite (*2*), as well as sulfur dioxide (*3*), have been used as sources of the sulfurous acid.

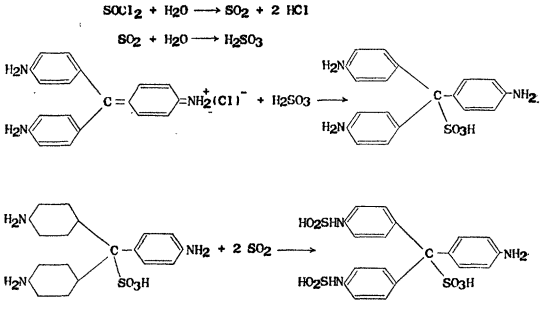

FIG. 1. Reactions which can be expected to occur when thionyl chloride is used as a source of sulfurous acid in Schiff's reagent.

The following method, in which thionyl chloride is used as the source of sulfurous acid, has been found to be both simple and effective. First, 1.0 gm of basic fuchsin is dissolved in 400 cc of distilled water. Second, 1.0 cc of $SOCl_2$ (thionyl chloride, CP) is added. The flask is stoppered and allowed to stand for 12 hrs. Third, after decolorization of the dye, the solution is cleared by the addition of 2 gm of activated charcoal, after which the mixture is shaken and immediately filtered (*1*). The solution is stored in a well-stoppered bottle.

It is likewise possible to treat the 0.25% basic fuchsin solution with charcoal prior to its decolorization and to obtain a clear, colorless solution on the addition of thionyl chloride.

In accordance with the work of Wieland and Scheuing, the reactions which occur should be as represented in Fig. 1.

The observed pH of Schiff's reagent prepared with thionyl chloride is 1.24; that of Schiff's reagent prepared in the usual manner is 1.38. This slight difference in acidity does not appear to be significant.

Schiff's reagent prepared with thionyl chloride has been successfully used as a nuclear stain on tissue sections of human thymus gland, kidney, liver, and spleen, as well as on the fungi, *Blastomyces dermatitidis* and *Saccharomyces cervisiae.*

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Plant Virus for Electron Microscopy

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The uniformity of size of an infectious unit has a significant bearing on the purity of a culture when the causative agent of a disease is to be established. Considerable variability in the size, particularly in the length, of tobacco mosaic virus particles, as seen in the electron microscope, has been reported. Oster and Stanley (*5*) have supported earlier conclusions with new measurements of virus obtained directly from leaf hairs of infected plants. Sigurgeirsson and Stanley (*6*) have also measured the virus particles in freshly expressed juice and found them more uniform than those in older or in centrifuged material. Later results (*3, 4*) show that the greatest number of virus particles (20–40%) fall into a group size of 15×280 mμ, and that less than 5% are extraordinarily short or long. It is emphasized by these workers that much of the variation in length is attributable to aggregative or disruptive forces occurring during preparation of the virus concentrate for examination under the electron microscope.

In connection with certain virus studies made in this laboratory, it has been found that the application of direct water pressure to plants systemically infected with mosaic yields clear exudation drops containing a sufficient virus concentration to be observed when applied to the electron microscope screens. The approximate concentration of individual droplets may be checked by wiping drops on a local lesion host with the tip of a finger or a glass spatula. This exudate contains some extraneous material; but, with the gold shadow-casting process, the virus particles were easily found in considerable numbers. In the case of plants with hydathodes, the virus may be exuded from these natural openings, but with other plants the droplets were collected chiefly from the cut ends of veins.

This method of obtaining a relatively pure form of virus was described from this laboratory in 1924 (*1*). The method has also been illustrated in other papers in connection with water-congestion studies (*2*). Where larger quantities of virus are required, as for purposes of concentration by differential centrifugation, a more recent modification has consisted of collecting the exuded drops in a 14″ metal side-arm funnel properly placed under the plant foliage. In this manner 25–50 cc may be collected in a few hours from suitable plants, especially if the leaf blades are cut so as to expose the ends of numerous veins. The virus concentration of this exudate is not usually as high as that secured by the usual procedure of expressed juice. However, practically all of the extraneous plant material that interferes with electron microscopy is eliminated. With the water-pressure method, the exuded droplets may be applied directly to the collodion film of the electron microscope screen. Drying in a desiccator, followed by gold shadow-casting, completes the shortened preparation for electron microscope examination. Several strains of the tobacco mosaic virus particles have been collected in this manner, and different host plants, especially tobacco, tomato, *Eryngium aquaticum* L., and *Digitalis lanata* L. (a previously unreported host species), have been used. The method is applicable to glaucous plants such as *E. aquaticum* and *D. lanata*, where the leaf-hair method would not be possible.

The pressure exudate method of preparation of the virus for electron microscopy should not cause aggregation or disruption of particles of the infectious unit, especially when the first drops are used, since the actual pressure at the point of exudation is very low. Variation from the normal virus length in the droplets, if such occurs, would necessarily seem to arise from the drying process on the screen. The latter supposition is discounted by those who have found the virus particle relatively uniform in length (*3*). Measurements of the virus particles obtained by the water-pressure method have not yet been made. The electron micrographs, however, show a high variability in the length of the particle, not approaching, for example, the uniformity exhibited by most cultures of microorganisms.

This method of obtaining virus may prove useful for other studies by means of electron microscopy.

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