in the refrigerator at +5° C, and samples were removed at intervals for the vaccination of mice, utilizing the technique of Habel and Wright (*4*).

TABLE 1

POTENCIES OF ANTIGENS EXTRACTED WITH VARIOUS SOLVENTS

Solvent used	Viability at 10^{-3} dil.	Antigen LD_{50} protection
Acetone	–	15,000
Benzene	+	290,000
Carbon tetrachloride	–	100
Dichlorethylene	–	3,000

The results of the first test of antigens extracted with various solvents are presented in Table 1.

The virus used in this experiment was not inactivated before extraction, and it was found after extraction that benzene had failed to kill all of the rabies virus. No viable virus was demonstrated at 10^{-3} dilution following extraction with the other solvents. Since benzene yielded the most potent antigen and was found to be least toxic to the virus, further experiments were undertaken using this solvent for extraction. It was determined that extraction as outlined above effected a marked reduction of the lipid content of the dry vaccine. The effect of cold benzene and ether on the titers of virus in two different lots is presented in Table 2.

TABLE 2

THE EFFECT OF DRYING AND OF BENZENE-ETHER EXTRACTION ON THE TITERS OF RABIES VIRUS

	Original infective titer	Infective titer after drying	Infective titer after extraction
1)	10,000,000	1,000,000	1,000
2)	501,000	13,000	400

Since extractions with benzene and ether in the cold did not completely inactivate rabies virus, subsequent extractions were done on mouse brain suspensions previously inactivated by ultraviolet light (*3*, *5*). In each case another sample of the same irradiated lot, dried but not extracted, was used as a control. In two experiments the dried, or dried and extracted, tissue was treated with phenol to kill the virus. The relative potencies of extracted and unextracted vaccines are compared in Table 3.

TABLE 3

COMPARISON OF THE POTENCIES OF EXTRACTED AND UNEXTRACTED, NONVIABLE VACCINES

Experiment No.	LD_{50} protection by control vaccines (unextracted)	LD_{50} protection by extracted vaccines
1*	0	36,000
2	10,000	274,000
3	10,000	274,000
4	568,000	568,000
5	4,000	400
6†	700	9,000
7†	100	9,000

* The source material was an outdated vaccine.
† Phenol killed.

It is evident that the antigenicity of extracted vaccines is as good as, or better than, that of unextracted material of the same lot in most instances. No explanation is offered for the observation, but the experiments listed, and others to be reported later, consistently suggest significant difference in favor of the extracted vaccines. In our hands, experimental phenolized vaccines have tended to be of lower antigenicity than corresponding irradiated vaccines.

It has thus been found that extraction of rabies vaccine with cold benzene followed by cold ether will remove much of the lipid (41.5% of the total dry weight) of vaccine. The process causes a marked reduction in the infective titer of rabies virus in brain tissue, but does not kill all of the virus under the conditions described. Rabies vaccines killed with ultraviolet light and extracted do not lose antigenicity, but in most instances are actually improved as antigens. As a result of our experiments we have concluded that benzene and ether-extracted vaccines may offer some advantage over unextracted vaccine for the prevention of rabies. However, the greatest advantage of lipid extraction is the elimination of one of the major nonspecific components of a very crude vaccine.

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A Constant-Temperature Micromanipulation Chamber

KENNETH M. RICHTER[1]

Department of Histology and Embryology, University of Oklahoma School of Medicine

Although the constant-temperature micromanipulation chamber to be described here was developed in connection with certain studies in progress on mammalian tissue, it, unlike the special constant-temperature apparatus described by Peterfi (*3*), has a wide range of application not only in this general field of investigation but also in other fields not employing the micromanipulative technique. This instrument was designed specifically for use with the Chambers micromanipulator (*1*), but its basic

[1] I would like to extend my thanks to E. F. Hiser, of our Medical Art Department, for the preparation of the illustrations, to E. R. Flock for certain technical suggestions, and to our Physiology Department for making its facilities available to me.

functional features may be adapted to those types of chambers required by other manipulators (*1*).

The instrument is constructed entirely of sheet Plexiglas, and the parts are welded together with Plexiglas solvent. The thermal, electrical, optical, and other properties of Plexiglas make it a material of choice. The apparatus consists essentially of two units: a manipulation unit (Fig. 1A) and a gas-mercury thermostat (B). The manipulation unit has an outside measurement of $2'' \times 3'' \times \frac{1}{2}''$ with an off-center moist chamber (C) of essentially standard dimensions (*2*, *4*). It consists of four welded, appropriately shaped plates: a floor plate $\frac{1}{16}''$ thick (D), a shell plate $\frac{1}{4}''$ thick (E), a step plate $\frac{1}{16}''$ thick (F), and a roof plate $\frac{1}{16}''$ thick (G). An inner chamber (H) bounds the moist chamber (C) on three sides. In operation, the inner chamber is filled with tap water. This chamber opens to the outside through an outlet nipple (I) to which is attached a small rubber overflow tube (J). The latter is directed toward the rear edge (K) of the unit and inserted into a tube support (L). Two threaded brass electrodes (M, N) extend for a short distance into the inner chamber through the left edge (O) of the unit. Each of these electrodes is sealed watertight by means of a rubber washer (P) under compression by a plastic nut (Q). A hole (R) in the left edge of the unit accommodating the activating chamber (S) of the thermostat is made watertight by the vertical block of the thermostat (T), which is welded directly to the edge of the unit.

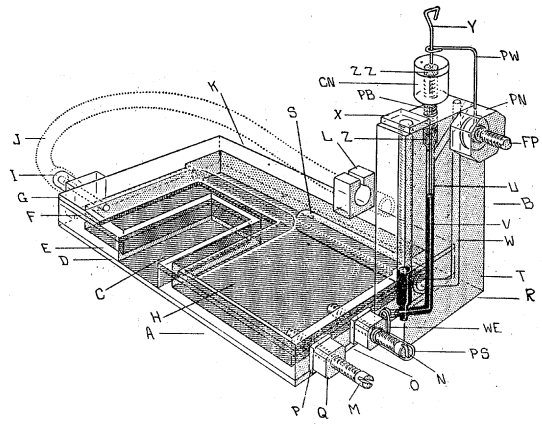

FIG. 1

The bulk of the thermostat consists of a vertical block (T) in which three vertical channels, appropriately interconnected, have been drilled. These channels together constitute an upright modified U-tube (U) partially filled with mercury (V) and a long side arm (W) connecting it to an activating chamber (S) filled with air and lying within the water filled inner chamber of the manipulation unit. The front arm of the U-tube is sealed airtight by a small plate (X). An adjustable platinum wire electrode (Y) for temperature regulation dips into the mercury column in the rear arm of the U-tube. It is sealed airtight by a rubber disc (Z) put under compression by a plastic bolt (PB) screwed into the upper end of this arm, which has been appropriately countersunk and threaded. In addition, it is sealed by means of a rubber disc (ZZ) compressed against the end of the plastic bolt by means of a plastic capping nut (CN). A fixed post (FP) for tying into the electric source consists of a brass bolt held by a recessed plastic nut (PN) welded to the side of the vertical block. A platinum wire (PW) connects this fixed post to the adjustable platinum wire electrode (Y). Another platinum wire electrode (WE), welded in position permanently, connects the mercury column in the U-tube with the rear electrode (N) of the manipulation unit. The exposed outer end of this latter electrode is insulated by means of a rubber or plastic sleeve (PS), and the wire is coated with shellac. Lead-in wires from a standard 110-volt a–c source are attached by means of insulated sleeve type connectors to the fixed post of the thermostat (FP) and to the front electrode of the manipulation unit (M).

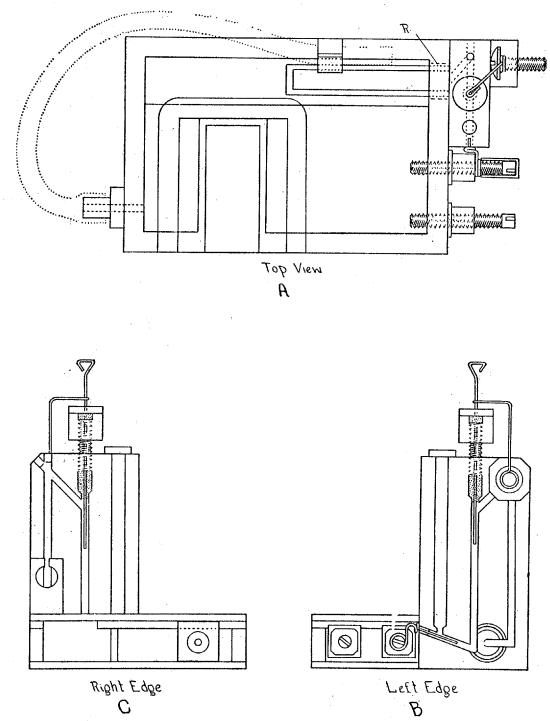

FIG. 2

The heating device consists simply of the passage of the electric current through the tap water filling the inner chamber by means of the manipulation unit electrodes (M, N). The heat generated is due to the electrical resistance of the tap water. The circulation of the water within the inner chamber depends on the formation of and the guidance of the convection currents established. The roof of the inner chamber is not of uniform height. Because of the step plate (Fig. 1F; Fig. 2A, B, C) it is low near the electrodes and higher near the far sides of the moist chamber. Consequently, as the water is heated, the warmer, and thus lighter, strata are guided almost directly into the far side of the inner chamber, making for

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

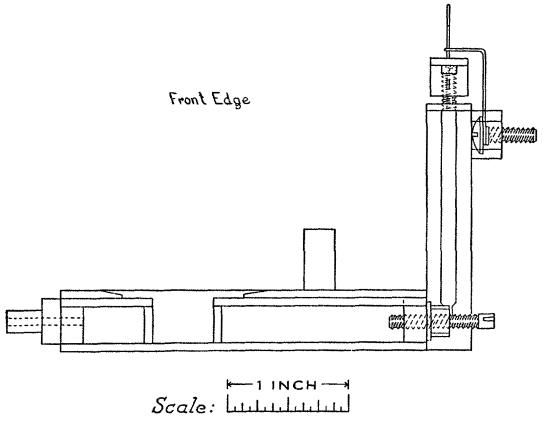

FIG. 3

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

JAMES D. BARGER
Mayo Foundation

EDWARD D. DELAMATER
Mayo Clinic, Rochester, Minnesota

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.

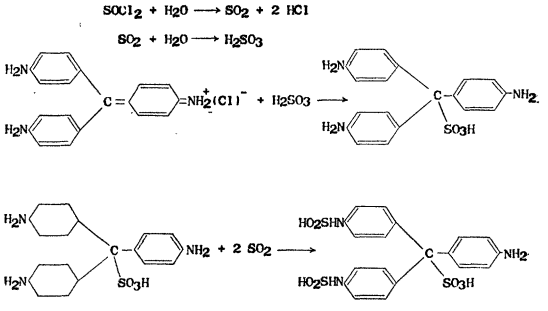

FIG. 1. Reactions which can be expected to occur when thionyl chloride is used as a source of sulfurous acid in Schiff's reagent.

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.