

and rate of air exchange, it would supply a volume of 1,300 cubic feet with 9 complete air changes per hour. With the vaporizer at room temperature, the vapors can be demonstrated above the apparatus within 2 minutes of turning on the switch of the incandescent bulb. When the apparatus was used in a room of 1,260 cubic feet, the glycol was dispersed to all corners of the room with sufficient rapidity to reach bactericidal concentration within 30 minutes, in the absence of any human activity except that of the operator.

A typical series of tests of the apparatus gave the following results: a 1:10 dilution of an 18-hour broth culture of Group C streptococci was sprayed into the air of a room $9 \times 9 \times 15.5$ feet, and the organisms were dried by mixing the output of the spray with many volumes of room air in a large bottle. The window was opened $1\frac{1}{2}$ inches, and the relative humidity was 32. After 10 minutes, the bacterial spray was discontinued, and one Petri plate was exposed for 5 minutes near each corner of the room, at table height. The glycol vaporizer was then attached to the electrical outlet. At various intervals thereafter the spraying of bacteria and the exposure of plates were repeated identically as in the case of the controls above. All plates were incubated for 48 hours at 37° C., and colony counts were then made. In this series of experiments the table supporting the bacterial spray and glycol vaporizer was near corner D and farthest from C (Table 1).

TABLE 1

CONTROL COLONY COUNTS AND COUNTS AT INTERVALS AFTER CONNECTING THE VAPORIZER TO THE ELECTRICAL OUTLET

Site	30 Minutes				60 Minutes			
	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
	Con- trol	With glycol	Con- trol	With glycol	Con- trol	With glycol	Con- trol	With glycol
A	339	28	280	20	99	1	252	3
B	322	14	272	10	97	1	238	2
C	280	37	252	14	87	0	220	1
D	374	3	208	3	94	1	231	1

Similarly satisfactory results were obtained on spraying *Escherichia coli* and *Staphylococcus albus*. The concentrations of bacteria in air produced by spraying in these tests are, of course, very many times the concentrations found by similar means in any normal habitation.

The application of this vaporizer is not predicated on the use of any particular glycol. At present there is no general agreement among all workers in the field as to the glycol of choice, and it is entirely possible that those in current use, propylene and triethylene, may be superseded. Propylene glycol was used in these experiments and seems at present to be preferable for the applications for which the device was in-

tended, *i.e.* small enclosed spaces not equipped with devices for regulating the rate of vaporization. The most important reason for this is that the wider range of concentration between bactericidal and precipitation thresholds in the case of propylene glycol obviates the necessity for special regulatory instruments.

Finally, no attempt was made, in developing this device, to provide means of controlling the relative humidity. Recent work by Hamburger, Hurst, Robertson, and Puck (3) has shown that, although the bactericidal effect of triethylene glycol vapor is greater in the presence of relative humidity above 40, it is only somewhat lower at a relative humidity of 18-30 per cent. Work done with propylene glycol in this laboratory is consistent with these findings. This minimum relative humidity is not higher than is necessary as a general hygienic measure and should be maintained by some means which is a part of the heating or ventilating apparatus. Hamburger, *et al.* suggest for the purpose steam caps in steam-heated interiors. In the apparatus described here the technical problems of simultaneous vaporization of glycol and water would defeat the original purpose of producing as simple a device as possible.

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Fine-tapered Silver Electrodes for Physiological Work¹

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In the course of electrophysiological studies of nerve activity in insects (1) fine and pliable electrodes were necessary. It was found that 28 to 36 B- and S-gauge silver wire was too coarse, while finer gauges were subject to whip and vibration. Conventional saline-filled glass capillary electrodes had the disadvantage of high electrical resistance, while it was impossible to bend such electrodes during an experiment to conform with the short nerves and narrow operational fields encountered in insects. Various mechanical methods of tapering silver wire were tried without success until a simple and rapid electrolytic method was developed.

Leads are soldered to short lengths of No. 28 B and S soft-drawn silver wire, which are mounted in a glass

¹ The electrodes were developed in the course of work done under a contract between the Chemical Warfare Service and Tufts College.

holder to be carried by the manipulator. A 50-ml. beaker is filled with 5 per cent aqueous silver nitrate in which a piece of silver wire is immersed. The latter is connected to the negative pole of a 6-volt d-c source (storage or dry batteries), while the leads from the electrodes are connected to the positive pole. The electrodes are rapidly immersed in the silver nitrate to a depth of about 2 mm. and immediately withdrawn. This is repeated four or five times, or until inspection under a microscope reveals tapered tips of the required shape and fineness. The angle of taper is determined by the speed of immersion and withdrawal, and can be varied accordingly. The silver remains soft and smooth and can be chlorided and insulated with shellac if desired. The great advantage of tapered silver electrodes is that the fine tips can be bent into any shape with fine forceps during the course of an experiment, and less than a minute is required to make new tapered tips, should the points break off.

The tendency to vibrate is slight since the electrodes can be made of relatively heavy silver wire, while the soft temper and fineness of the points greatly minimizes tissue damage which may occur when the electrodes are moved.

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Use of Sulfuric Acid-Dichromate Mixture in Cleaning Glassware

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Concentrated sulfuric acid saturated with potassium or sodium dichromate has been used for many years in cleaning glassware. In 1934 Laug (3) found that, although 10 rinsings of glassware with water removed all dichromate from the glass surface, there were still appreciable amounts within the glass, and that if the glass were allowed contact with water for several hours, it would yield the dichromate to solution. For example, in one experiment with a pyrex beaker 1.3 $\mu\text{g.}$ of potassium dichromate was removed. Only by boiling it out with several successive changes of water could the dichromate be removed effectively. Richards (4) studied the effects of such small concentrations of dichromate on yeast and other microbial forms and found as little as 0.0001 $\mu\text{g./ml.}$ to be toxic in some cases.

Because of the general use of this cleaning solution in most biological as well as chemical laboratories, it was considered of some importance to investigate further the retention of dichromate and the acid com-

ponent by glassware and the effect of dichromate on certain representative laboratory procedures.

Sulfuric acid-dichromate cleaning solution was made up in the usual way (250–300 ml. saturated potassium dichromate plus approximately 3,500 ml. technical concentrated sulfuric acid) and was found to contain 28,100 $\mu\text{g.}$ potassium dichromate/ml. (determined by iodimetry, using a solution of sodium thiosulfate standardized against potassium iodate). An extremely sensitive test for dichromate (1) entails the addition of diphenylcarbazide in an acid medium. The absorption curve of the resultant color product was determined on the Beckman spectrophotometer and a maximum extinction (E) observed at a wave length of 540 $\text{m}\mu$. Working at this wave length, a curve relating concentration to E was established by which all subsequent analyses for dichromate were determined. By this method, as little as 0.01 $\mu\text{g./ml.}$ can be detected. Determinations of pH were made using the Beckman pH meter.

Determinations of dichromate and pH were made on washings from a 5-ml. pyrex volumetric pipette, a 25-ml. pyrex volumetric flask, and a 250-ml. pyrex volumetric flask. The first 10 washes were done as rapidly as is usually done when washing glassware under a running faucet, and each washing was tested for pH and dichromate. In each case 6 to 10 rinses were required before the pH approximated that of the wash water. Four to 6 rinses removed most of the dichromate, although even after 10 rinses a small amount could be detected in some cases, e.g. the 10th washing from the 5-ml. pipette contained between 0.10 and 0.20 $\mu\text{g.}$ As would be expected because of the relatively large glass surface to its contained volume, more rinses were required for a pipette than a flask. To demonstrate the quantity of absorbed dichromate not removed by such rapid washing, a 250-ml. pyrex volumetric flask was allowed contact with cleaning solution for 48 hours. The 11th rapid wash contained 0.1 $\mu\text{g.}$ dichromate. The flask was then filled with water and allowed to stand for 22 hours at room temperature, after which the wash was concentrated to 10 ml. by heat evaporation. This wash contained 0.2 $\mu\text{g.}$ dichromate.

The effect of dichromate on urease activity was determined by adding various amounts of dichromate solution to the reactants in a modified Karr method for determining urea nitrogen.¹ Dichromate in the range of 1–10 $\mu\text{g./ml.}$ attained as much as 95 per cent inhibition of the enzyme urease. Since a direct relationship exists between enzyme concentration and concentration of inhibitor required for a specific amount

¹ A 1 per cent solution of Squibb's urease was used. The resulting ammonium carbonate solution (after reaction for 30 minutes at 55° C.) was nesslerized by Koch-McMeekin's reagent. The color intensity was measured at a wave length of 425 $\text{m}\mu$ on the Coleman spectrophotometer.