Automated Dehydration of Small Specimens[1]

L. J. STOWELL and MICHAEL A. McCLURE[2]

Ethanolic dehydration of biological specimens for electron microscopy is a standard technique. With specimens visible only under a dissection microscope, however, problems arise in handling. Transfer of tissues from one solvent to the next is required in conventional automated tissue preparation for microscopy. Confinement of the specimen becomes particularly difficult when it is smaller than 1 mm[3]. A device described by Viglierchio and Maggenti (1) substitutes solvent exchange for conventional tissue transfer in handling small specimens such as nematodes. Their solvent exchanger is suitable for preparation of large numbers of specimens. The specimen container is so large, however, that it is difficult to locate processed samples consisting of only one or a few nematodes. The S&M (Stowell & McClure) model 1000 programmable solvent exchanger, in contrast, has a low-volume glass specimen chamber in which the specimens can be inspected with a dissecting microscope (2). This chamber is coupled to the solvent exchanger, and ethanolic solutions of decreasing water concentration are pumped through the chamber (Fig. 1). Reproducibility of specimen dehydration is important for critical microscopy. In addition to reproducibility, low cost and a low-volume specimen chamber (2) make the S&M model 1000 programmable solvent exchanger superior to commercially available devices.

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[1] Journal Paper No. 2892 of the Arizona Agricultural Experiment Station.

[2] Respectively Teaching Assistant and Professor, Department of Plant Pathology, University of Arizona, Tucson, Arizona 85721. The authors are grateful to J. L. Tiss for technical assistance in circuit design.

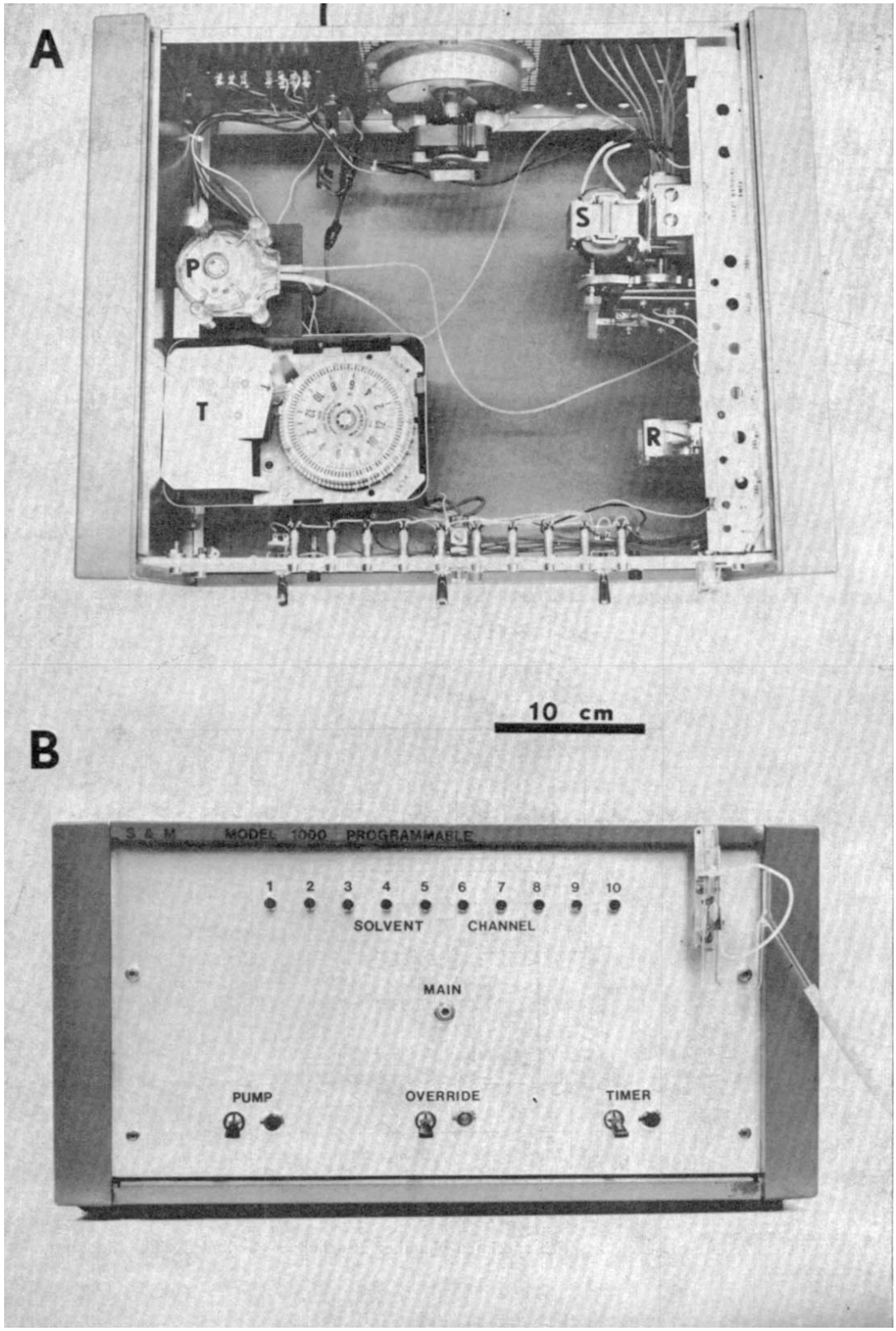

FIG. 1 (A-B). The S&M model 1000 programmable. A) Top view showing the pump (P), timer (T), solenoid (S), and relay (R); B) front view.

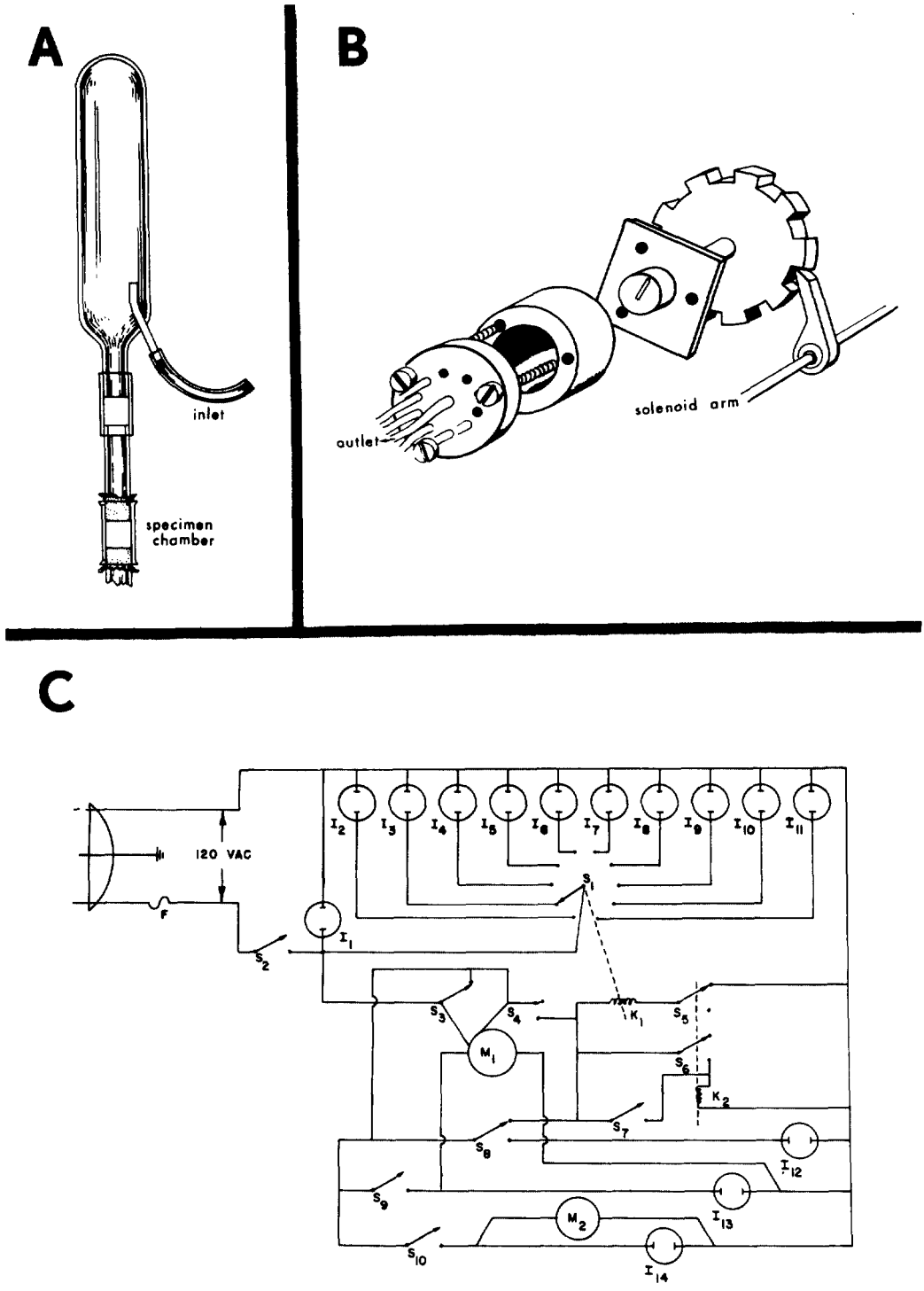

FIG. 2 (A-C). A) Mixing chamber with specimen chamber attached; B) exploded view of rotary valve; C) circuit diagram.

Solutions of ethanol are stored in ten separate reservoirs. A Teflon tube connects each reservoir to a solenoid-actuated rotary valve constructed of Teflon. Selection of the solvent reservoir is controlled automatically by a timer or manually by an override switch. Solvent is drawn from one reservoir at a time through the valve (Fig. 2B) by a peristaltic pump at a rate of 0.8 ml/min. The solvent is then pumped into a 2.0-ml mixing chamber (Fig. 2A). This device produces a series of gentle step gradients by mixing solvents in the 2.0 ml of dead volume of the mixing chamber. Ethanol has a density of about 0.8 g/ml. Therefore, mixing occurs as lower-density solutions are pumped into the bottom of the mixing chamber. About 4 min are required to change the solvents in the mixing chamber at a flow of 0.8 ml/min. The rate of specimen dehydration is determined by the length of time each solvent is pumped through the specimen chamber and the change in water content between successive reservoirs. This prototype was constructed in 1977 at a cost of under $300.00 (parts only).

The electrical relationships of the components are described in Fig. 2C. The component designations of the schematic (e.g., S_1) are in parentheses following the component name. The products listed were chosen by specification and availability. Any comparable substitute would be satisfactory.

Pump (M_2): Peristaltic, Cole Palmer Inst. Co., Chicago, Illinois 60648. Five-rpm drive unit, catalog no. 7543-05. Head model no. 7014.

Solenoid (K_1): Intermittent-duty Dormyer solenoid. W. W. Granger, Inc., Tucson, Arizona 85719. One-inch stroke, 6.0 lb pull per inch, 23.0 watts at 115 VAC, 60 Hz. Stock no. 4X898. Manufacturer's no. 7110-2A.

Relay (K_2, S_5, S_6): Dayton double-pull, double-throw relay. Dayton Electric Mfg. Co., Chicago, Illinois 60648. 120 VAC, contacts rated 12 amps resistive load. Manufacturer's no. 5X838.

Timer (M_1, S_4): Dayton 24-hour clock. Single-pull single-throw switching action. Dayton Electric Mfg. Co., Chicago, Illinois 60648. 20 amp at 125 VAC. Manufacturer's no. 2E026.

Main switch (S_2, I_1): Push-on, push-off, with neon light (I_1). Radio Shack, Fort Worth, Texas 76107. 6 amp at 250 VAC. Catalog no. 275-671.

Microswitch (S_3): Unimax standard lever microswitch. Mounted on top of the factory-installed timer switch S_4. W. W. Granger, Inc., Tucson, Arizona 85719. Catalog no. V3L-1108-D8. Unimax no. 2TMT15-4.

Microswitch (S_7): Unimax microswitch. S_7 is actuated by Solenoid K_1 at the top of its stroke. W. W. Granger, Inc., Tucson, Arizona 85719. Catalog no. BZ-2RW80-A2. Unimax no. 2HBT-5.

Toggle switch (S_8, S_9, S_{10}): Single-pull, single-throw toggle switch. Radio Shack, Fort Worth, Texas 76107. 6 amp at 250 VAC. Catalog no. 275-654.

Indicator lights (I_2-I_{11}): Neon lamps.

TABLE 1. Position of switches required for different operations by the dehydrator.

System status	Switch no.								
	S_2	S_3	S_4	S_5	S_6	S_7*	S_8	S_9	S_{10}
Machine off	+[1]	+	+	+	+	+	−	+	+
Normal operation between timer trippers	−[2]	+	+	+	+	+	−	−	−
Normal operation on timer tripper	−	+	−	−	−	−	−	−	−
Normal operation automatic stop	−	−	+	+	+	+	−	−	−
Manual selection of solvent channel using override (S_8)	−	+	+	−	−	−	+	−	−

[1] + indicates the switch is in the position shown in Fig. 2C.
[2] − indicates the switch is in the alternative position to that shown in Fig. 2C.
*Switch S_7 only operates momentarily when K_1 reaches the top of its stroke.

Radio Shack, Fort Worth, Texas 76107. Built-in dropping resistor 120 VAC. Catalog no. 272-703.

Indicator lights (I_{12}-I_{14}): Neon lamps. Radio Shack, Fort Worth, Texas 76107. Built-in dropping resistor 120 VAC. Catalog no. 272-705.

Switch (S_1): This switch was constructed at the University of Arizona. It is suggested that a 12-way rotary valve be used in constructing similar devices; 12-way rotary switches are commercially available; 10-way rotary switches are not.

Rotary valve: The 10-way rotary valve used in this prototype was constructed at the University of Arizona. Commercially available valves are suitable for this application. A 12-way ceramic-faced rotary valve is available from JZ Associates, Brookline, Massachusetts 02146. Model no. RV1.

LITERATURE CITED

1. VIGLIERCHIO, D. R., and A. R. MAGGENTI. 1965. Automatic solvent exchanger. Trans. Am. Micros. Soc. 84:284-293.
2. McCLURE, M. A., and L. J. STOWELL. 1978. A simple method for processing nematodes for electron microscopy. J. Nematol. 10:376-377.