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POLYVINYL ALCOHOL-FIXATIVE-ADHESIVE FOR SMALL HELMINTHS AND PROTOZOA

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Following the method of Brooke and Goldman (1949), the author was able to fix, mount and stain small helminths and parasitic protozoa of fish in polyvinyl alcohol-fixative smears. However, because there was much granular material in the PVA-Schaudin's mixture, AFA fixative was substituted for the Schaudin's. The PVA went into solution readily using this fixative. The solution was accomplished by adding 5 grams Elvanol #71-30¹ to 62 ml. of water, heating to 95° C. until dissolved and adding 5 ml. of acetic acid, 1½ ml. of glycerine, 10 ml. of formaldehyde, and 30 ml. of 95 percent alcohol.

Protozoa were placed on a slide in a drop of water; 2 drops of PVA-AFA were added and the mixture spread over approximately one-half of the slide. The slide was dried two days at room temperature or overnight in the 55° C. oven. This mixture appeared to fix *Trichodina*, *Myxosoma* spores, *Oxytricha*, and *Paramecium* as well as the PVA-Schaudin's mixture.

After drying, the slides were stained, dehydrated and mounted as ordinary smears. In no instance did the smears come off. As Brooke and Goldman (1949) noted, the slides can be re-dried at any stage before dehydration prior to mounting.

As many live cercariae as could be collected in a drop were placed on a slide and two drops of the PVA-AFA mixture added. This was spread out and allowed to dry as for protozoa. Cercariae of *Posthodiplostomum m. minimum* and *Crassiphiala bulboglossa* were fixed in a slightly contracted state but were satisfactory for staining. The slides were stained and mounted with very little loss of material. Previously fixed cercariae can be handled in the same manner. Those fixed in hot 5-10 percent formaldehyde and then attached to the slide with PVA should yield excellent specimens.

Small metacercariae and nematodes that are so easily lost during handling were fixed in a small Petri dish or beaker of hot 10 percent neutralized formaldehyde, transferred to a slide in a drop of fixative, and one drop of PVA-AFA was added. Slides were dried, stained, and mounted as before. The metacercariae of *Uvulifer*, *Crassiphiala*, *Diplostomulum*, *Ornithodiplostomum*, and the small nematode, *Rhabdochona*, were easily handled by this method. Hot AFA fixative caused the worms to stick to the container and they were damaged in removal. However, hot formaldehyde alone did not cause them to stick but did prevent extreme contraction. Small helminths can be fixed directly in the PVA-AFA solution on a slide, but they contract as they do in any cold fixation. Attempts to anesthetize the worms in 0.4 percent chloretone previous to mounting in the PVA-AFA solution also resulted in contracted speci-

¹Courtesy of Electrochemicals Department, E. I. DuPont deNemours Co., 7 S. Dearborn Street, Chicago 3, Illinois.

mens. Dropping hot PVA-AFA onto the worms on a slide likewise resulted in contracted specimens. Trematode ova were badly distorted when mounted directly in cold PVA-AFA.

REFERENCES CITED

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