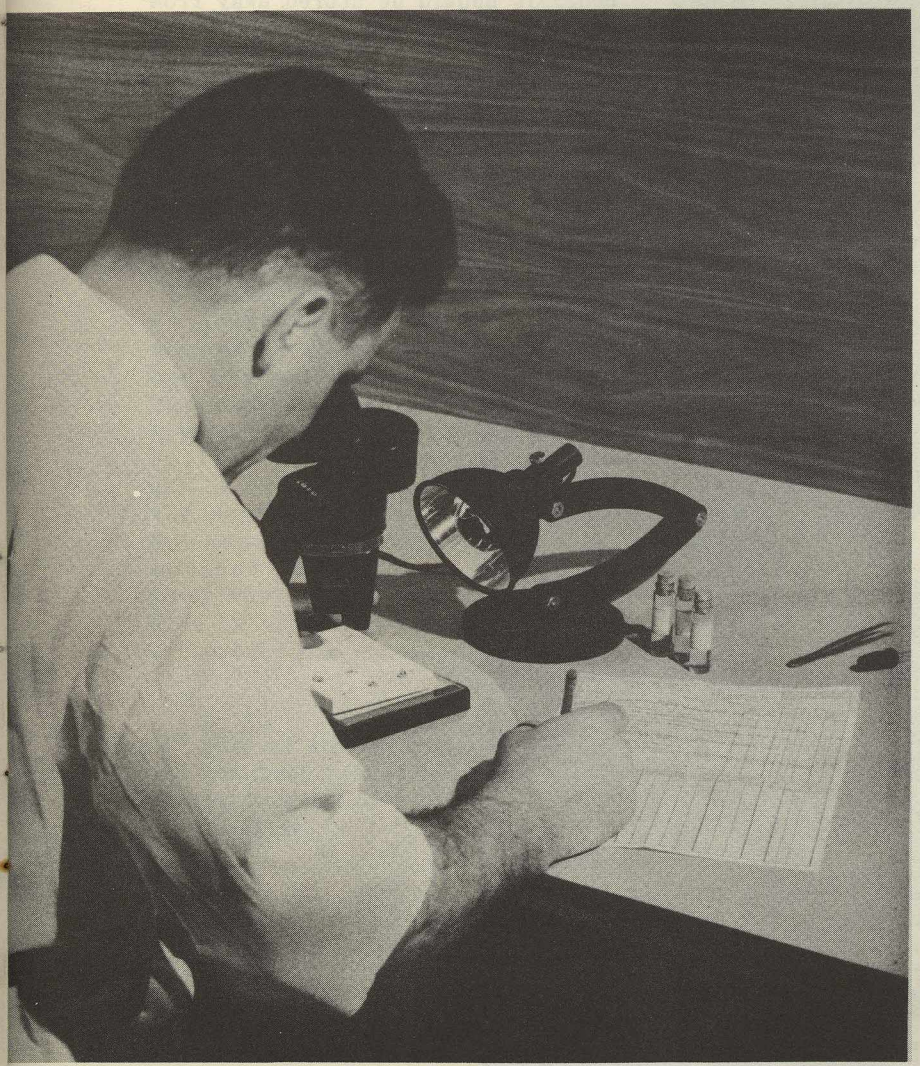
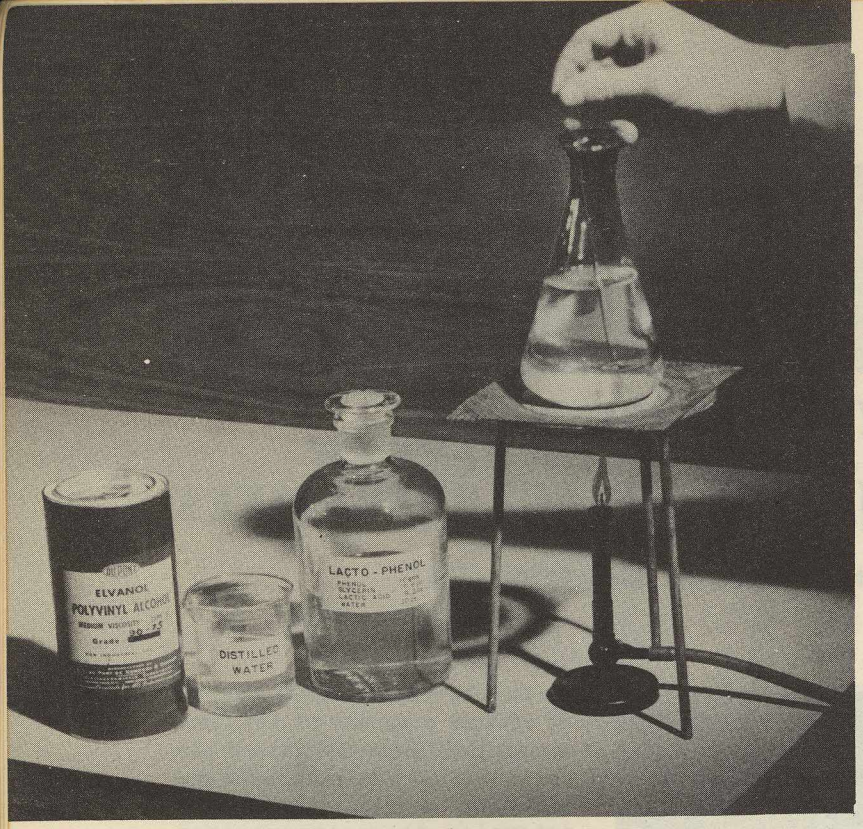
POLYVINYL ALCOHOL FOR ROUTINE DETERMINATION OF ECTOPARASITES

Harry D. Pratt and John E. Lane



As On the cooperative typhus control programs of the Communicable Disease Center and the various State Health Departments, it is relatively easy to determine the ectoparasites commonly collected on domestic rats. The majority of such specimens usually represents only six species of fleas, four species of mites, and two species of lice which have well-marked distinguishing characteristics. Usually it is possible to determine 95 percent or more of the specimens simply by pipetting them out of the vials onto a white porcelain depression plate and examining them with a dissecting microscope. However, as the Communicable Disease Center and State Health Department programs expand to include research and demonstration control programs for such diseases as sylvatic plague and the arthropod-borne encephalitides, a much larger group of ectoparasites is encountered, including many species of fleas, ticks, mites, and sucking and biting lice from a wide variety of birds and mammals. The specific determination of this much larger group of ectoparasites then becomes a more difficult and time-consuming process requiring special mounting techniques.



B Polyvinyl alcohol (PVA) provides a quick, single-solution, semipermanent mounting media for the routine diagnosis of these ectoparasites*. It is made by adding 6.3 grams of PVA** to 35 cubic centimeters of water in a 500 cubic centimeter flask, stirring constantly. When all the PVA has been added, a milky suspension results which will clear up upon heating in a water bath to 75° C. While the solution is still warm, 45 cubic centimeters of Lacto-Phenol*** are added and stirred well. When the solution is cool, it should be stored away from light in a dark bottle.

*Bryn Jones — Impregnating polyvinyl alcohol with picric acid for the simultaneous staining and mounting of Acarina. Proc. R. Ent. Soc. London (A) 21, Pts. 10-12: pp. 85-86 (Dec. 1946).

**DuPont "Elvanol" — Medium viscosity — Type B, Grade 90-25. The use of this does not represent an indorsement of the product by the Public Health Service.

***Lacto-Phenol is made by mixing 10 grams of phenol with 10.6 cubic centimeters of glycerin, 8.2 cubic centimeters of lactic acid, and 10 cubic centimeters of distilled water.

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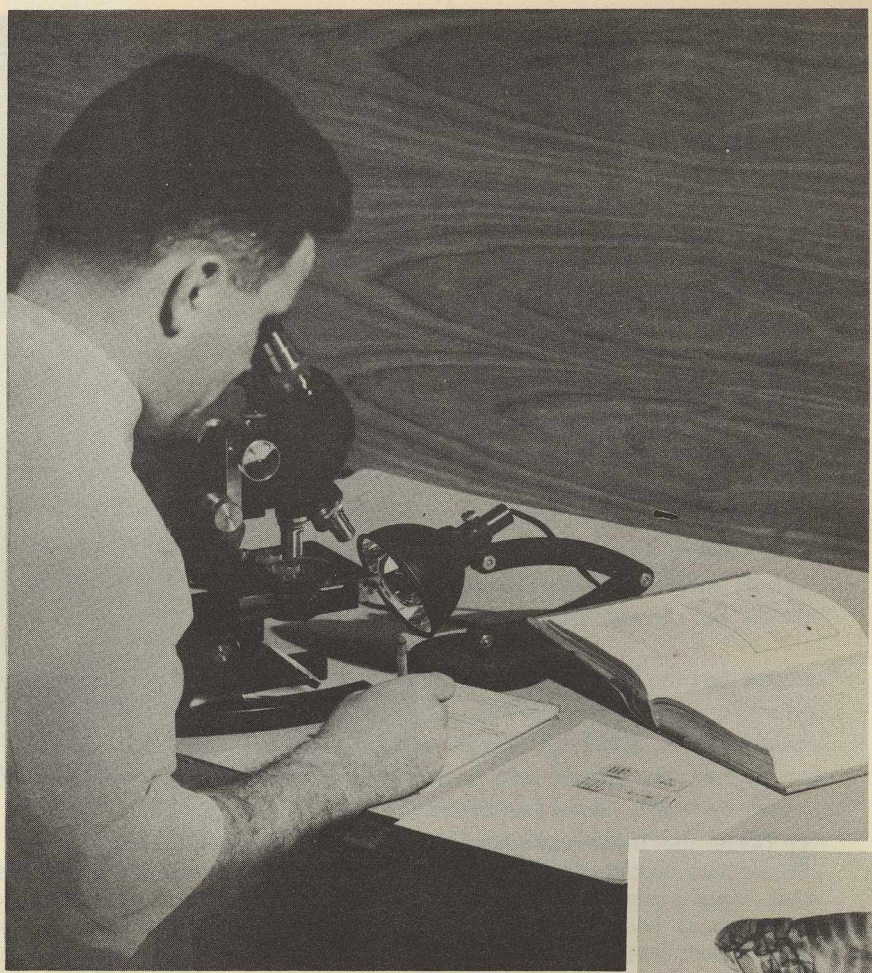
C The specimens submitted from the field, either in 70 percent alcohol or water, may be mounted directly in PVA-Lacto-Phenol. It is usually easiest to do this by pipetting all the specimens onto a white porcelain depression plate. Then, enough PVA-Lacto-Phenol is spread on the slide to cover an area equivalent in size to the cover glass. The specimens are placed in the smear of PVA-Lacto-Phenol in regular rows with fine forceps or a wire loop mounted in an applicator stick. A cover glass is placed gently on the smear. If the smear is moderately viscous and of sufficient size, very little movement of the specimens will occur beneath the cover glass.



D If the collection is small, as many as 40 or 50 specimens may be mounted on a single slide and the collection data reference number marked with a wax pencil. If the collection is large, it may be necessary to mount the specimens on several slides. The fleas are mounted upside down so that they appear right-side up when seen through a compound microscope.

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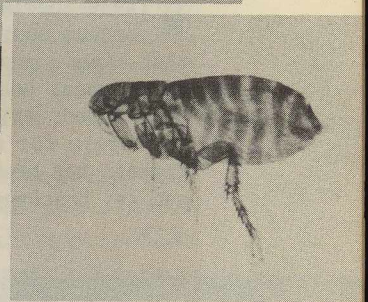
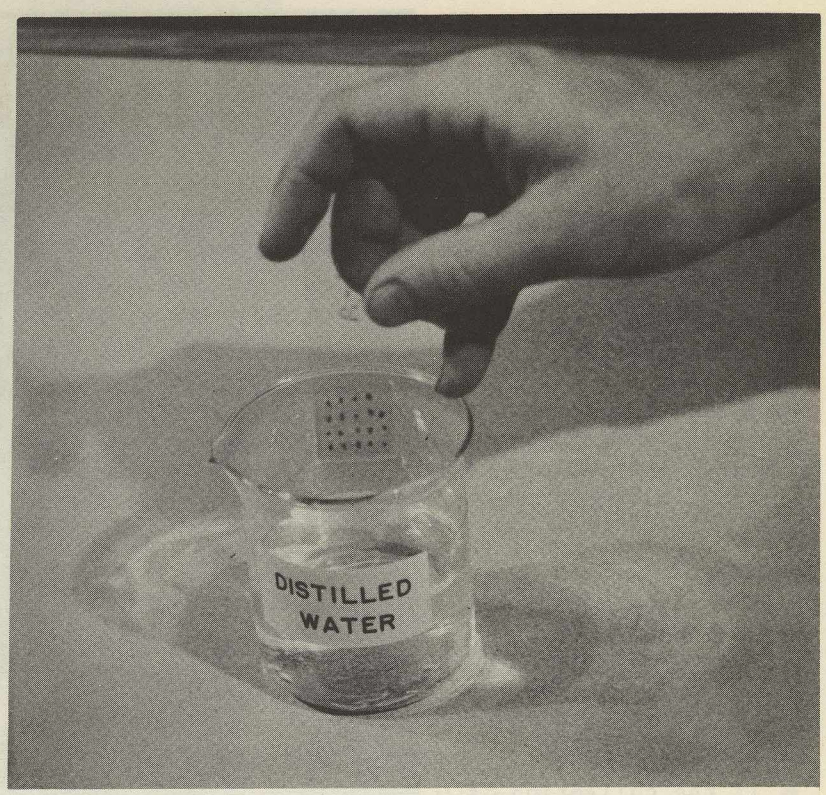


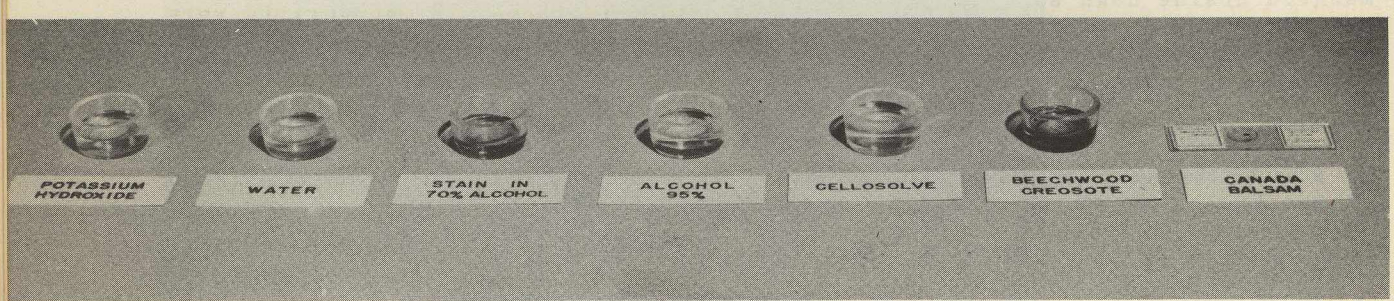
E Many of the specimens, especially the fleas, may be determined as soon as the cover glass has been placed on the smear. Some specimens, such as chiggers or mites, are easier to determine a day or two later after they have been cleared by the action of the PVA-Lacto-Phenol on the slide.

F Specimens which are cleared show the fine detail used in classification, such as the number and arrangement of hairs or combs on fleas. Slides with common species have been stored for months in the laboratory without deterioration, although other slides show a tendency toward clouding or production of air bubbles under the cover glass 3 to 6 months after the slide has been made.

G If rare or unusual specimens are found on slides mounted in PVA-Lacto-Phenol, they may be recovered by soaking the slide in water at room temperature overnight, or in an oven at 65° C. for a few hours. This dissolves the PVA and frees the specimen from the slide.



H These specimens may then be mounted by the usual long method: (1) soak in 5 percent or 10 percent sodium or potassium hydroxide solutions until clear, usually pressing the specimen with a bent needle or fine forceps during this process to expel the dissolved tissue; (2) wash for several hours or a day in water to which a few drops of 15 percent hydrochloric acid has been added; (3) dehydrate in 70 percent alcohol, or in the case of mites and lice, dehydrate and stain for a day or two in 70 percent alcohol to which a few drops of 0.5 percent acid fuchsin has been added; (4) dehydrate further and clear in 95 percent alcohol, (5) cellosolve, and (6) beechwood creosote; and (7) finally mount in Canada balsam.



I Such specimens can be appropriately labeled and stored as a part of a permanent reference or study collection. These slides should show the finer details of hairs and structures used in classifying these ectoparasites.