CONVENTIONAL methods for the preparation of small arthropods for study under the microscope are both tedious and time consuming. Furthermore, when fine structural detail has to be clearly shown, resource has usually to be made to treatment with caustic potash; a procedure that is always fraught with a certain amount of risk to the specimen, particularly if the latter is a small insect of the order of one tenth of a millimetre long. To a taxonomist working on the study of large quantities of biological material any reduction in the time spent in preparing his specimens for study would be a great boon. For these reasons I had, for some time prior to 1947, been paying considerable attention to this problem and in 1946 had my attention drawn to the possibility of using polyvinyl alcohol as a mounting medium in microscopy. This material was first used as a substitute for cover glasses in clinical work by American investigators. It was later used in conjunction with Amman's Lactophenol as a mounting medium both in England and America but the formulae published frequently refused to set or the resulting mounts were spoiled by a granular precipitate. However, there seemed to be possibilities in the material and I commenced a series of experiments which resulted in the development of some six different mounting media that I was able to announce to the Sixth Science Congress of the Royal Society of New Zealand in 1947. (1.)

Polyvinyl alcohols are sweet smelling, creamy-white powders and the mounting media prepared from them are characterised by their crystal clearness, low refractive indices and by the fact that they will not crystallise as so often happens with gum mounts of the Berlese type. But the great advantages of these polyvinyl alcohol media lie in their ability to replace the tedious conventional mounting procedures, to clear biological material almost perfectly and in their tolerance of a wide variety of fluids. Material can be mounted direct into them from ethyl alcohol of any strength, from Andre's Fluid, from Pampel's Fluid, from MacGregor's Solution, from formalin, from water, from glycerin, from lactic or acetic acids or from life. In this respect I think that they are unique. After the media have ripened there is practically no shrinkage whatever in specimens: in fact, the tendency is for these media to extend specimens and there is no need for undue haste in placing the cover-glass; the operator may take his time. I have left delicate
specimens on slides in Type A.3 of these media, uncovered, for up to half an hour without harm; with conventional resinous media the pressure from the placing of the cover glass is often necessary to prevent shrinkage.

All these media completely relax small insects in a few seconds and much can be done to arrange a specimen in any desired position by gently "rolling" it with the cover-glass, even to the extent of completely turning it over by gentle motion of the cover in the desired direction. In addition they all have a very intense clearing action on biological material, and it is seldom necessary to resort to potash treatment of specimens. This clearing action is strongest with Type A.1 and Type MA.2. To hasten the hardening of the mount and to assist in this clearing action slides should be held in a paraffin embedding oven or incubator at about 50° C. for about eight hours. After this treatment they may be examined quite safely with an oil immersion lens. This heat treatment tends to drive out any trapped air bubbles and, if desired, may be considerably speeded up by gently warming on a spirit stove until ebullition occurs and all air bubbles have been driven out. This latter procedure also brings about a much more rapid setting of the mount which can then be examined almost immediately afterwards, with reasonable care, but it does, however, have a tendency to rupture very delicate material. This applies particularly to material mounted direct from life or water and which has not been toughened by immersion in alcohol for at least twenty-four hours beforehand.

Clearing is exceedingly rapid with all Type A and MA formulae, but with Type B it is much slower and continues for 24-36 hours. It is never so intense with Type B as with Type A. Deep pigments in specimens are rendered quite transparent, and partially bleached, while delicate pigments are destroyed. Alkaline stains are unstable in these media, but acid fast stains are perfectly satisfactory. The medium undergoes a slight yellowing in the bulk with ageing but the mounts are colourless and the specimens thrown up with a boldness and relief which is really remarkable.

These media have now been used by me in my studies of Collembola for over four years with extremely satisfactory results. They have also been used by others for mounting such things as thrips, mosquitoes and their larvae, mites, spiders, opiliones, pseudoscorpions and rotifers with the same outstanding results in each case.

To prepare a set of these six formulae it is necessary to procure some light viscosity polyvinyl alcohol of the types Elvanol A. 51.A.05, Elvanol B. 70.A.05 and some medium viscosity polyvinyl alcohol of the type Elvanol 71-24. Prepare 120 c.c. of lactophenol by adding 60 grams Phenol Detached Crystals B.P. to 60 c.c. of Lactic Acid B.P., stir until dissolved warming gently if desired on a water bath. The polyvinyl alcohol is difficult to dissolve in water and this is best done in each case by adding the water, drop by
drop, to the powder, stirring it into a thick paste. When all the powder is wet the remainder of the water may be added. The resulting "paste" is fluffy and white due to occluded air and may be cleared by gentle heating on a water bath. Provided that all the powder is wet and in solution there is no danger in adding the lactophenol to this fluffy solution. If the powder is not all properly in solution, insoluble lumps will form when the lactophenol solution is added. The lactophenol solution is added in each case with vigorous stirring and the resulting mixture cleared by heating on a water bath. It is then ready for use. When freshly prepared there may sometimes be effervescence from specimens transferred from water, and the media display a momentary intolerance of alcohol, but both these effects disappear as the media age and ripen. They should be stored away from strong light.

**Formulae:**

**Type A.1.**
- **Refractive Index 1.469**
- Elvanol 51.A.05 2.5 grams
- Distilled water 6 c.c.
- Lactophenol solution 30 c.c.

**Type A.2.**
- **Refractive Index 1.458**
- Elvanol 51.A.05 2.5 grams
- Distilled water 10 c.c.
- Lactophenol solution 25 c.c.

**Type A.3.**
- **Refractive Index 1.447**
- Elvanol 51.A.05 2.5 grams
- Distilled water 15 c.c.
- Lactophenol solution 25 c.c.

**Type B.**
- **Refractive Index 1.438**
- Elvanol 70.A.05 2.5 grams
- Distilled water 10 c.c.
- Lactophenol solution 15 c.c.

**Type MA.1.**
- **Refractive Index 1.425**
- Elvanol 71-24 2.5 grams
- Distilled water 20 c.c.
- Lactophenol solution 25 c.c.

**Type MA.2.**
- **Refractive Index 1.442**
- Elvanol 71-24 2.5 grams
- Distilled water 15 c.c.
- Lactophenol solution 30 c.c.

In preparing these formulae it is necessary to measure all quantities accurately. Any variation from these amounts may cause a granular precipitate to appear in the finished preparation, or the media may fail to set. If a precipitate should appear it may be removed by adding additional lactophenol solution drop by drop, with stirring, until the precipitate disappears; the crucial point at which this occurs should be detected by examining samples under the microscope. If too much lactophenol is added the medium will not set. If the media become too viscous during the course of continued use this is the result of evaporation of water and can be rectified by the careful addition of a few drops of distilled water. As the water is added a white precipitate will form but this redissolves on stirring and the medium returns to its old viscosity.

On completion these media are colourless, crystal clear, relatively viscous oily liquids. The most useful formulae I have found to be
Type A.2 and Type MA.1 for all general purposes. Types A.1 and MA.2 are extremely useful for clearing and extending old or shrivelled specimens: I have used them successfully to clear and extend insect specimens that had been preserved in alcohol for 84 years; no other treatment was necessary and the resulting mounts are perfect. Type A.3 is most useful for extending the appendages of small insects. Specimens left in it for 15-30 minutes become fully extended without the necessity for manipulation with needles, after which the cover-glass may be carefully placed in position and the mount left to harden. Within this range of six formulae is a series of media offering varying powers of clearing and extending specimens and different degrees of refractive index. Intelligently used they can be of considerable use with different classes of material.

REFERENCE