"Celochloral" — a New Mounting Medium for Insects

By

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For mounting soft-skinned insects, as aphids, modern entomologists have generally kept to media containing gum arabic and chloral hydrate, and, in addition, either glycerine (Faure's gum chloral) or glucose and acetic acid (Berlese's gum chloral). These mounting media have certain disadvantages, among these a tendency of a fine granulation appearing in the mounts after some months or years. With most formulas, sealing is necessary to prevent air creeping into the mounts from the edges of the cover-glass, ultimately filling the mounts with ramifying ducts.

Various compounds on the basis of polyvinyl alcohol have proved more or less definite failures owing to their generally very strong reduction of volume after evaporation of water. After some time, mounts with these media are often depressed to an undesirable extent, and air will be forced into them from the margins of the cover-glass. As remounting is very difficult with polyvinyl media, the use of these may lead to deplorable losses of insect material. I have made some 4,000 mounts with various polyvinyl alcohol media. These looked very fine for some months, but after one or two years most of them must be regarded as more or less valueless.

Fortunately, however, certain other media tried by the present author seem to be more reliable. One is "Celodal", a medium manufactured by Bayer in Leverkusen, Germany, and recommended for mounting macroscopic biological objects. This medium is a colourless, water-soluble, very viscous fluid. After diluting with some water, it may be used also for microscopical mounts of insects and mites, especially for strongly sclerotized and well pigmented specimens. After maceration in KOH or lactic acid, specimens may be transferred into the mountant directly from water, alcohol or lactic acid. (On "Celodal" as a medium for microscopical mounts, see Feltz, 1953, and Ant, 1957.) For more delicate insects, as aphids, the refractive index of "Celodal" is a little too high, however. For aphids, coccids, aleyrodid pupa cases

and similar insects I use "Celochloral", a medium with the following formula (which is, as is easily found out, a Berlese medium with "Celodal" replacing the gum arabic):

"Celodal I" 100 g
Chloral hydrate 120 g
Glucose 20 g
Glacial acetic acid 20 ml
Water 100 ml.

Dissolve the chloral hydrate and the glucose in the water at room temperature, add the acetic acid and filter. Add the "Celodal" and mix by stirring. The mixture should be allowed to rest for some hours before use, in order to give air bubbles in the viscous fluid time to disappear. If a stock of this mountant is stored for a period longer than a few months, small rhomboid crystals may sometimes appear. In order to avoid including these crystals into the mounts, the mountant should be stored in high narrow flasks, so that the crystals may settle on the bottom of these. These crystals will not appear in the mounts if not introduced when they are being made. "Celochloral" should not be diluted with more water. Mounting directly from water is not advisable, as a milky cloudiness in the mounts will most often appear if this is done.

Insects or mites which are advantageously cleared by treating with lactic acid can be transferred into "Celochloral" directly from concentrated or nearly concentrated acid. If treatment with KOH is preferable, the procedure proposed by Hille Ris Lambers (1950) can be used. This procedure, slightly modified, can be briefly described as follows:

A. Rapid clearing procedure

1. If the insects are fresh, heat them cautiously with some 95 % alcohol in a test tube in the waterbath for some minutes. Violent boiling of the alcohol should be avoided. If the specimens have been stored for some time in alcohol, heating in alcohol is unnecessary.

2. Decant the alcohol. Boil the specimens for 1—5 minutes, depending on the size of the insects, with some 10 % KOH in the waterbath. After this treatment, the specimens should be semitransparent. If maceration is incomplete, the insects will shrink after mounting.

3. Add some 95% alcohol, and the specimens will sink to the bottom of the test tube. Decant the liquid.

4. Add some 95 % alcohol containing a small amount of acetic acid. Boil gently 1—2 minutes (waterbath). This is done in order to prevent the subsequent appearing of bubbles of CO_2 in the mounts. Decant the fluid.

5. Heat with a few ml of chloral phenol, a saturated solution of chloral hydrate in phenolum liquefactum, for 5—10 minutes (boiling waterbath). After this treatment the specimens should be quite transparent.

6. Mounting can be done immediately, or the insects may be stored in chloral phenol until the mounting can be conveniently done.

Small specimens may be transferred directly into "Celochloral" from chloral phenol. Large amounts of phenol should not be introduced into the "Celochloral", however, or a milky turbidity will sometimes appear during the drying of the mounts. Moreover, the acid content of the medium will revert the saponification effected by KOH, so that more or less disturbing drops of fatty acids will appear in the bodies of some specimens. Therefore, as much as possible of the fluid content of the bodies should be pressed out, at least from large specimens, and such specimens should be soaked in a saturated solution of chloral hydrate in water or alcohol before mounting. Dry the mounts during 24—48 hours at 60°C in a thermostate.

This method has the advantage of working very fast. The most important disadvantage is the difficulty of removing fat drops from the interior of the insect bodies. Personally, therefore, I rarely use this method, preferring the following procedure, which however takes several days.

B. Slow clearing procedure

The insects are treated with the various chemicals at room temperature and in darkness. They are kept in glass tubes 8 cm in length and with an inner diameter of 6 mm. The lower end of each tube is closed by a piece of fine-mesh cotton gauze kept in place by a glass ring fitting round the tube. The cotton gauze endures 10 % KOH at room temperature (but not 10 % NaOH!). The tubes can stand upright in narrow glass cylinders containing the various fluids. They can be conveniently moved from one cylinder to the next one, by which the transferring procedure of the insects is much simplified. I treat my aphids with the following fluids:

1. A mixture of equal volumes 95 % alcohol and ethyl ether, 24—48 hours.

2. 95 % alcohol, 24 hours.
3. 10 % KOH, 24(—48) hours, depending on the size of the insects.
4. Water, 24 hours.
5. 95 % alcohol with some acetic acid added, 24 hours.
6. Chloral phenol, 24 hours or longer, until mounting can conveniently take place.
7. Saturated solution of chloral hydrate in water or alcohol, a few minutes or more.

Before mounting, the glass ring is removed from the tube and the piece of cotton gauze with the insects is placed in a small Petri dish or similar vessel with some chloral hydrate solution. If the specimens are large, excess fluid should be blotted off with a slip of filter paper before transferring into the mountant. Air bubbles in the mountant or in the insects should be removed by aid of a pin before covering with the cover-glass. Dry the mounts 24—48 hours in a thermostate at a temperature of about 60°C.

I have made some 4,300 mounts with this medium, all without sealing. The oldest are a little less than 4 years old. No changes of any kind have been observed in these mounts. The medium in the mounts seems to retain its elasticity for years.

Dissolution for remounting is difficult with this medium, however it can be done by keeping the slides for a few hours in hot water or hot 10 % KOH, suitably in a thermostate.

References

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