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Euparal as a Permanent Mounting Medium for Helminth Eggs and Proglottids

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The importance of euparal as a permanent mounting medium for eggs of parasites and the process of making permanent slides are analyzed and discussed in detail. Studies show that helminth eggs mounted in euparal exhibit excellent optical and drying properties. Euparal also can be used to identify proglottids of *Taenia* species and to examine for the presence of *Trichinella spiralis* in suspicious muscle biopsies.

Several types of mounting media are in vogue for preserving materials permanently for microscopic examination. These mountants are classified into three groups according to the treatment the material receives before mounting. They are (i) those that are miscible with water, such as glycerol, (ii) those that combine with alcohol and to which objects may therefore be transferred after dehydration but without having to pass through a clearing agent, and (iii) the conventional resins and balsams to which objects can be transferred only after they have been dehydrated and cleared (4).

This paper deals with a medium that has been in use as a permanent mountant for insects in general and medically important ones such as mosquitoes in particular (1, 2, 5). This medium, known as euparal, belongs to the second group of mountants described above. Euparal, which is a neutral mountant, is used for substances that are considered too delicate to preserve in balsam or other resins. Euparal is readily miscible in alcohol, but it also has a preparatory substance of which the composition is secret. This substance is available through Flatters and Garnett Ltd., Manchester, England, or GBI (Labs) Ltd., Heaton Mills, Denton, Manchester, England. Two types of euparal are available on the market, clear (yellowish) and green; the green color is due to the addition of copper salts.

One of us (O.G.W.B.) has been using euparal as a permanent mounting medium for the last 2 decades in the preservation of various stages of mosquitoes. All insects, like other arthropods, are characterized by the chitinous cuticle, and euparal, like balsam, makes them optically better for microscopic examination. It is known that all helminths are diagnosed by the presence of cuticle in the adults and larval stages. They are mounted in either balsam or Permount. Therefore an attempt was made to ascertain the efficacy of using euparal as a permanent mountant for both flat and round worms.

MATERIALS AND METHODS

Most helminth eggs recovered in the clinical laboratory are fixed in either polyvinyl alcohol or Formalin. Both fixatives are a good starting point for making permanent slides. The procedure is as follows.

(i) Transfer a drop of well-mixed feces preserved in polyvinyl alcohol or Formalin to a slide and spread it uniformly (size of a dime).

(ii) Dry the slide in an incubator set at 50°C for 5 to 10 min.

(iii) Leave the slide in a Coplin jar with 70% ethanol for 5 to 10 min; transfer to 90% ethanol (3 to 5 min) and finally to absolute alcohol (5 to 10 min). The time frames can vary; longer duration leads to better dehydration.

(iv) Transfer the slide from absolute alcohol to xylene (3 to 5 min) or Cellosolve (glycol monoethyl ether; about 20 to 30 min). The use of Cellosolve allows slower dehydration, thereby minimizing shrinkage of eggs.

(v) Overlay with 2 or 3 drops of euparal, add a cover slip, and leave the slide in the incubator for 24 to 48 h for drying.

While studying the various helminth eggs, it was also decided to observe the effect of euparal on tapeworm proglottids. Gravid and immature proglottids were placed in absolute alcohol for 5 to 10 min, then pressed between two slides to flatten them uniformly, and finally mounted in euparal. Transparency occurred in 2 to 3 h, but better results were obtained by keeping the slides in the incubator overnight.

RESULTS AND DISCUSSION

Studies on insects and other arthropods have shown that euparal has better optical and drying properties than balsam or Permount. It also has several other advantages. First, it is easily miscible with (among other substances) ethanol, nearly all essential oils, a special euparal essence, and Cellosolve, which is also miscible with water.

Therefore it is possible to make a euparal permanent mount with only one or two steps directly from water. Second, the objects can be manipulated or dissected in euparal before the cover slip is laid. Third, since euparal is miscible with alcohol, euparal essence, or xylene, it is easy to remount the specimen (especially proglottids) if the slide or cover slip is broken.

Helminth eggs. All helminth eggs (with the exception of *Hymenolepis*) exhibited excellent optical properties when examined under the microscope. The cuticle became extremely transparent, and the internal details were exceptionally clear. We studied eggs of *Ascaris lumbricoides* (Fig. 1), *Diphyllobothrium latum* (Fig. 2), and *Paragonimus westermani* (Fig. 3). The medium was not very satisfactory for *Hymenolepis* species because the eggs shrank.

Tapeworm proglottids. Several species of cestodes are important from the standpoint of human health. The strobila or proglottids are shed periodically in feces. They are best studied as stained whole mounts, but to facilitate rapid identification, a temporary mount can be prepared by injection of India ink. If the proglottid belongs to the genus *Taenia*, treatment may depend on the species identified. Extreme caution should be used when handling the segments, since the eggs of *Taenia solium* are infective to humans through aerosols (3).

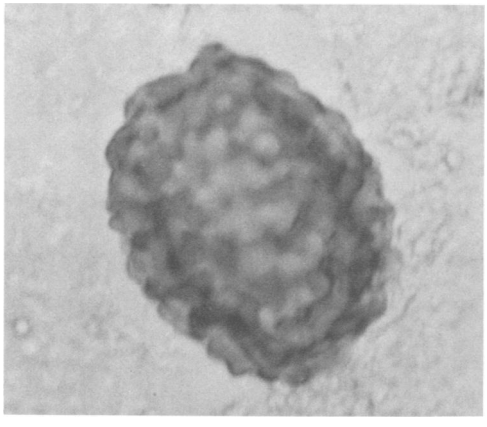

FIG. 1. *Fertilized egg of Ascaris lumbricoides (magnification, 500×; high dry objective).*

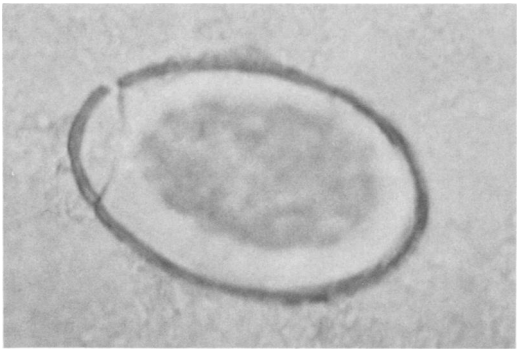

FIG. 2. *Egg of Diphyllobothrium latum (magnification, 500×; high dry objective).*

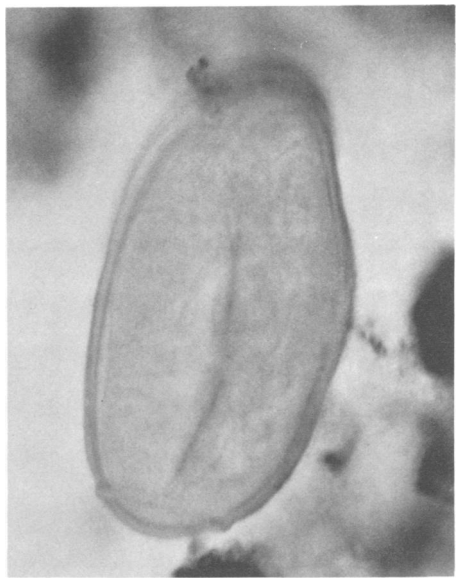

FIG. 3. *Egg of Paragonimus westermani (magnification, 500×; high dry objective).*

There are definite advantages to mounting proglottids in euparal. First, it takes only 2 to 3 h before definite identification is made. Second, the species can be identified even when the segment is immature. The identity of species is based on the number of branches in the uterus. The branches are distinctly seen with a stereoscopic microscope (Fig. 4). Third, one can make permanent slides without staining. Finally, the method is safe because there is no chance of creating an aerosol.

Trichinosis infection. Even though the larvae of *Trichinella spiralis* are found in striated muscles, they can easily be missed on casual examination. When suspicious muscle biopsies are mounted in euparal, however, it becomes easy to identify the encysted worms because the muscle fibers become extremely transparent. The whole procedure can be completed within 1 to 2 h.

An unfavorable property of euparal is its tendency to shrink material (1), but since helminth eggs are small and compact, the shrinkage is minimal (exception *Hymenolepis*). Another disadvantage is the cost of the medium: euparal is more expensive than Permount. The other ad-

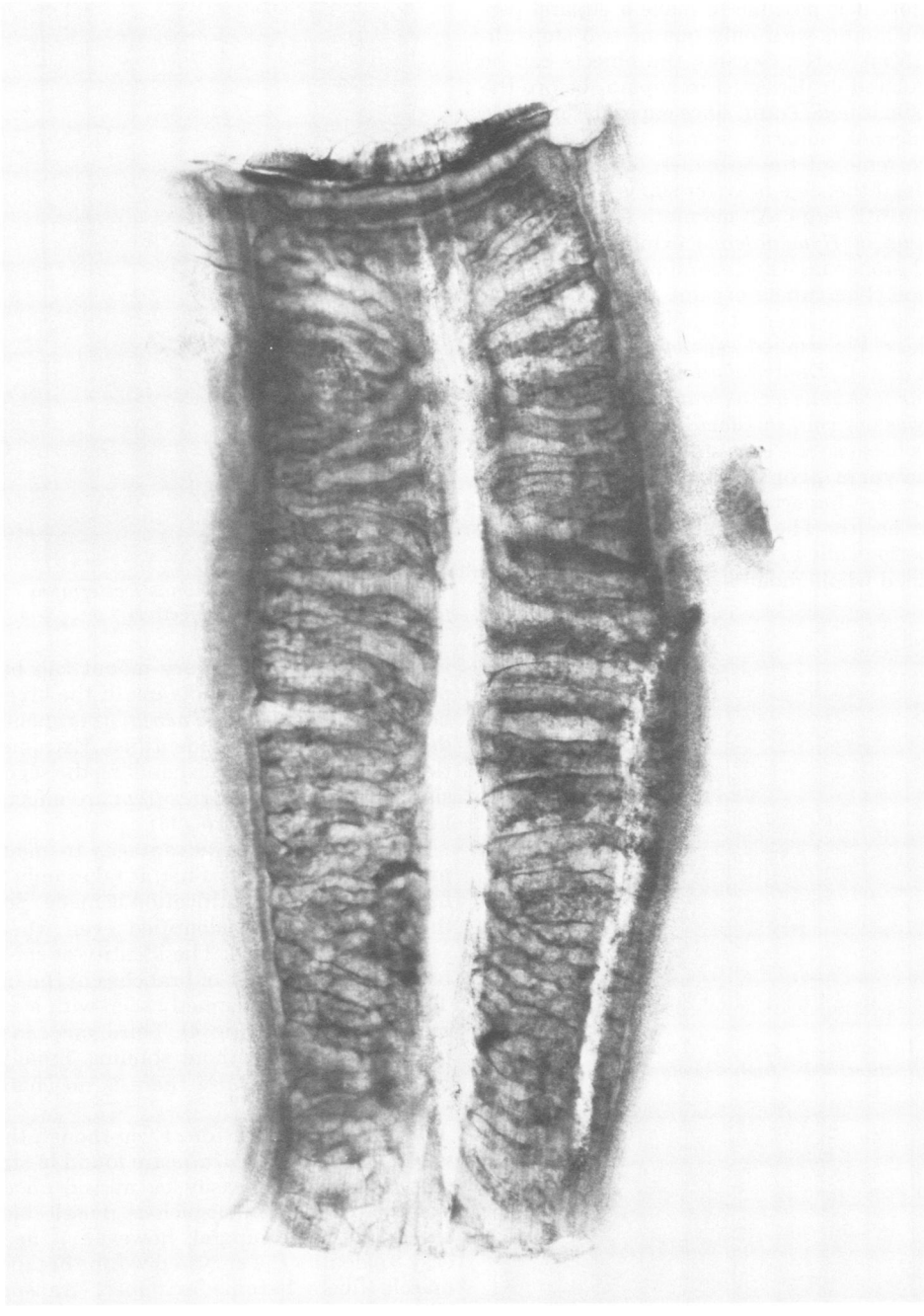

FIG. 4. *An immature proglottid of Taenia saginata, beef tapeworm.*

vantages definitely outweigh these defects, however. Finally, laboratories, large or small, can keep a reference collection of all clinically important parasites.

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