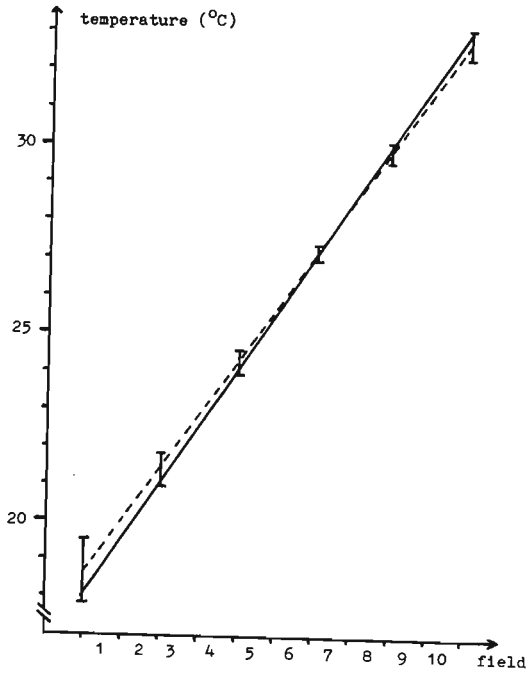

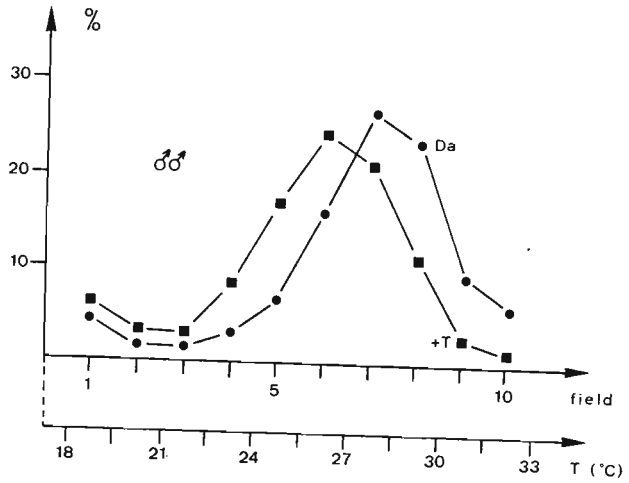

Fig. 3. Distributions of males of two strains of D.melanogaster along the temperature gradient (average of 12 replicates).

Fig. 2. Temperature profiles measured in the center (solid line) and at the margins (dotted line) of the apparatus. The temperature gradient in the center line is very stable and deviations are within the precision of the thermometers (±0.1°C). Vertical bars indicate maximum deviations measured at the margins of the aluminum sheet.

In each replicate about 50 anaesthetized flies are introduced into the center of an observation chamber and are allowed to distribute according to their preferences for about an hour. After this period carbon dioxide flows into the chambers and the flies become immobilized immediately and their number per field is counted. Figure 3 demonstrates the frequency distributions of males of a European and an African population from Berlin (+T) and from Benin (Da) along the temperature gradient. Each distribution is an average of 12 replicates.

Reference: Fogleman,J. 1978, DIS 53:212-213.

Kambysellis, M.P. New York University, New York USNA. A highly efficient method for collection of hemolymph, hemocytes or blood-borne organisms from Drosophila and other small insects.

Manual collection of hemolymph from individual Drosophila is time-consuming, the yield is low, and this method is often frustrating. An alternative method published previously (Kambysellis 1978), although faster and yielding higher volumes, has the disadvantage that unless extreme care is taken, the crop and the gut can be ruptured, and their contents together with cell debris are often obtained along with the hemolymph.

A modified method is presented here for collecting high yields of hemolymph, including the hemocytes, and yet free of other contaminants. The hemolymph is collected by centrifugation in an assembly adapted from the techniques of recombinant DNA work (deBruijn & Lupski 1984). We use two different size Eppendorf centrifuge tubes which precisely fit inside each other, a 0.5 ml tube and a 1.5 ml tube. With a hot needle (gauge #23), we open a small hole in the bottom of the smaller tube, which is then packed with glass wool up to the end of the narrow part of the tube (Fig. 1). The large amount of glass wool provides a cushion for the flies and prevents the squeezing of the flies during centrifugation which often leads to rupture of the crop. It is advisable but not essential to siliconize the glass wool prior to use. The tube with glass wool is then inserted into the larger tube which

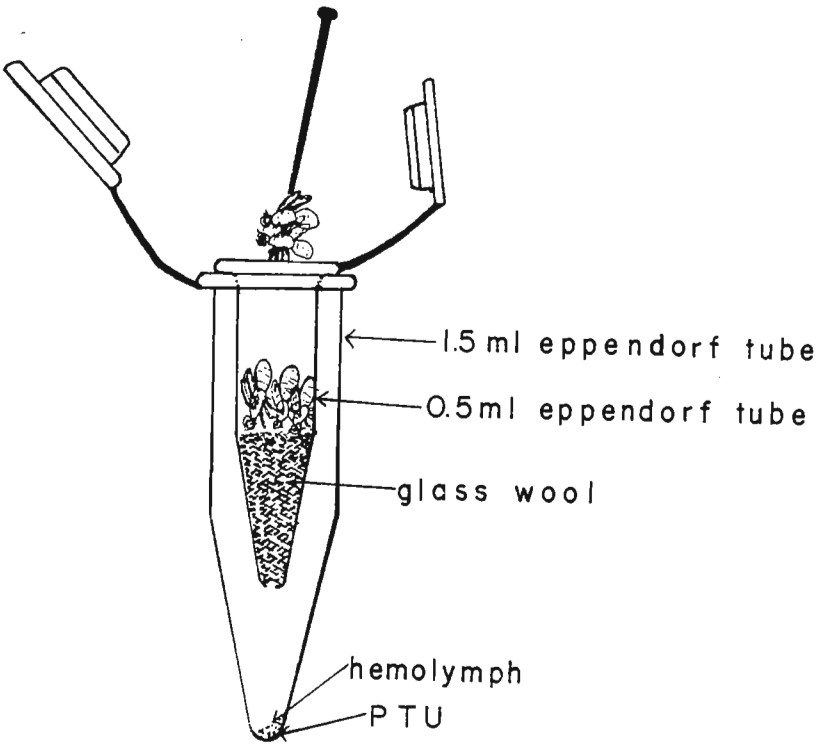

FIGURE 1.

contains 2-3 crystals of phenyl thiourea (PTU) to prevent tyrosinase activity in the hemolymph (Fig. 1). This assembly can now be wrapped in aluminum foil and sterilized if the material which will be collected is to be used for tissue culture work.

When ready to collect the hemolymph, the assembly is placed on ice for 10 min to cool. Under a dissecting microscope, the anaesthetized flies are stabbed in the thorax with #5 forceps or an insect pin, several at a time, and released with another forcep or pin directly into the smaller tube (Fig. 1). It is important in this step not to injure the abdomen of the fly, because the crop or gut will be ruptured and the hemolymph will become contaminated. It is also important to work fast and keep the apparatus cool to prevent protein denaturation in the hemolymph due to the presence of proteolytic enzymes, and also to reduce the chances of sealing of the wound by the hemocytes. We have found that for certain proteins (e.g., vitellogenins and serum proteins), even these precautions are not sufficient to prevent some degradation of the proteins. The degradation products although not detectable on stained SDS-polyacrylamide gels, became apparent in our immunological studies with Western blots, in which picogram quantities of vitellogenins (20-30 pg) can be detected. In such cases, degradation can be prevented by placing a few microliters of a proteinase inhibitor on the glass wool. (We routinely use 5 µl of 1mM phenylmethylsulfonyl fluoride.) If hemolymph protein concentrations are to be determined, then of course the appropriate allowance for the dilution with the inhibitor should be made.

When 20-40 flies are accumulated in the tube (this takes about 5 min), the assembly is placed in a refrigerated centrifuge and centrifuged for 3 min at 3,000 rpm. If a refrigerated centrifuge is not available, you can use a table-top centrifuge, the buckets and adaptors of which have previously been cooled on ice or in the refrigerator for 15-20 min. After centrifugation, the small tube with the flies is removed and discarded if not needed. (We routinely use these flies to dissect out ovaries or extract DNA.) The supernatant hemolymph is then carefully removed from the large vial so that the pellet of hemocytes and any organisms circulating in the hemolymph is not disturbed. The pellet is then resuspended in 100 µl of tissue culture medium or insect Ringer's for washing, followed by recentrifugation for 2 min at 2,000 rpm. The culture medium is then removed and discarded and the cells harvested in the appropriate buffer for a particular experiment. The duration and speed of centrifugation and the number of washings required to free the cells of hemolymph varies for different cell types and the optimal conditions should be determined in pilot experiments.

We have successfully used this technique to collect hemolymph from a variety of Hawaiian Drosophila species (Kambysellis et al. 1984), and to isolate the sex-ratio organisms from several other Drosophila species (Williamson & Kambysellis, unpubl. data). This method has also been adopted in malaria work to isolate Plasmodium knowlesi sporozoites from the salivary glands of decapitated infected mosquitoes (Ozaki et al. 1984).

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References: deBruijn & J.Lupski 1984, Gene, in press; Kambysellis 1978, DIS 53:218; ____, P.Hatzopoulos & E.M.Craddock 1984, W.Roux's Archiv, submitted; Ozaki,L.S., R.Gwadz & G.N.Godson 1984, J. Parasitology, submitted.