Bircsak, Edmund and Dennis O'Brian.
Seton Hall University. A procedure for obtaining nanoliter samples of haemolymph from Drosophila melanogaster.

Utilizing a variation of the method developed by Felix and Salceda ("A technique for microinjection in Drosophila" DIS; 39:135; 1964), a technique has been developed in this laboratory whereby it is possible to measure accurately nano quantities of haemolymph extracted from individual Drosophila. This technique initially involves the calibration of the inner bore of a sacrificed thermometer by transferring into this bore, with the aid of a Clay Adams suction apparatus under 5x magnification, a two microliter volume of mercury from a volumized Pasteur disposable pipette. The tip of the pipette was previously reduced by means of a microflame so that it would concentrically fit within the thermometer cavity.

After a series of calibrations, it was determined that the volume between any two successive one-degree marks represented 30.3 nanoliters. The calibrated thermometer was then connected to a Neptune Dyna minivac type pump by means of a glass T-join and rubber tubing. Known volumes of mercury could then be taken up and dispensed by merely constricting the diameter of either the vacuum or pressure tubes respectively.

Haemolymph extraction was done under a 40x magnification by inserting a drawn out glass micropipette into the haemocoel of the larva. By capillarity, the haemolymph entered the micropipette and the level of rise was indicated by a dab of india ink. The pooled or individual samples of haemolymph were then stored for future analysis.

Once the haemolymph was collected, its volume could readily be determined by dispensing known volumes of mercury from the calibrated thermometer into the extraction micropipette. The results are summarized in Table 1.

This method is applicable to any insect form except that the adult stage requires a prepuncturing of the chitinous exoskeleton to facilitate insertion of the micropipette into the haemocoel.

Utilization of other calibration methods such as that described by Prager, Bowman and Vurek (Science; 147:606; 1965) require specialized apparatus which may not be readily available in the ordinary laboratory. Moreover, without a silicon-carbide cutter a serrated tip results when the micropipettes are broken into convenient sizes. However, this source of error is eliminated in the method described in this note in that the fluid nature of mercury accomodates any serrated portion of the micropipette tip and insures accurate calibration, while the serrated tip also penetrates more easily into the haemocoel of the insect. Finally, the ease of preparing the micropipettes and their calibration still favors the use of individually calibrated pipettes.

Table 1: Extremes of variation in collecting nanoliters of haemolymph from third instar larvae of Drosophila on two separate occasions. Mean values are given with the standard error.

Number of trials	Number of units on thermometer		Volume in nanoliters	
	A	B	A	B
1	6	2	181.8	60.6
2	5	3	151.5	90.9
3	5	4	151.5	121.2
4	5	7	151.5	212.1
5	4	5	121.2	151.5
6	3	7	90.9	212.1
7	5	9	151.5	272.7
8	6	5	181.8	151.5
9	6	4	181.8	121.2
10	5	5	151.5	151.5
11		5		151.5
Mean			151.5±9.05	154.3±17.57