An efficient method of hemolymph collection from adult *Drosophila*

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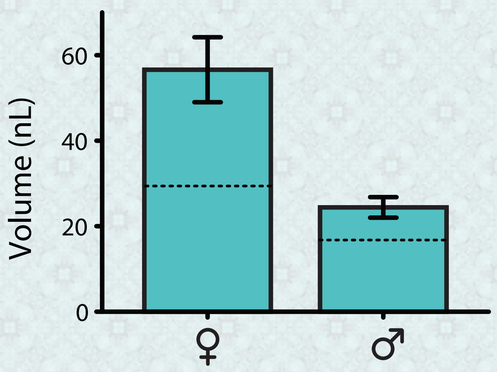
Introduction

- Hemolymph samples from small insects are needed for studies of ionoregulation, endocrinology, toxicology, and immunology.
- Current methods of hemolymph collection from *D. melanogaster* are slow and expensive[1], require anaesthesia[2,3], or yield low hemolymph volumes[4].

Here we describe a method for rapid collection of large volumes of hemolymph from adult *Drosophila* without anaesthesia.

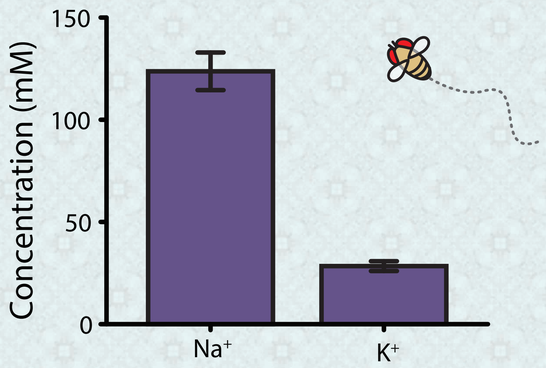
Extractable volume



Hemolymph volumes obtained from *D. melanogaster* using this method. *n*=10 flies/sex.

(---) Mean volumes reported by Piyankarage et al.[3] (extracted from anaesthetized flies using a custom silica capillary.

An example application



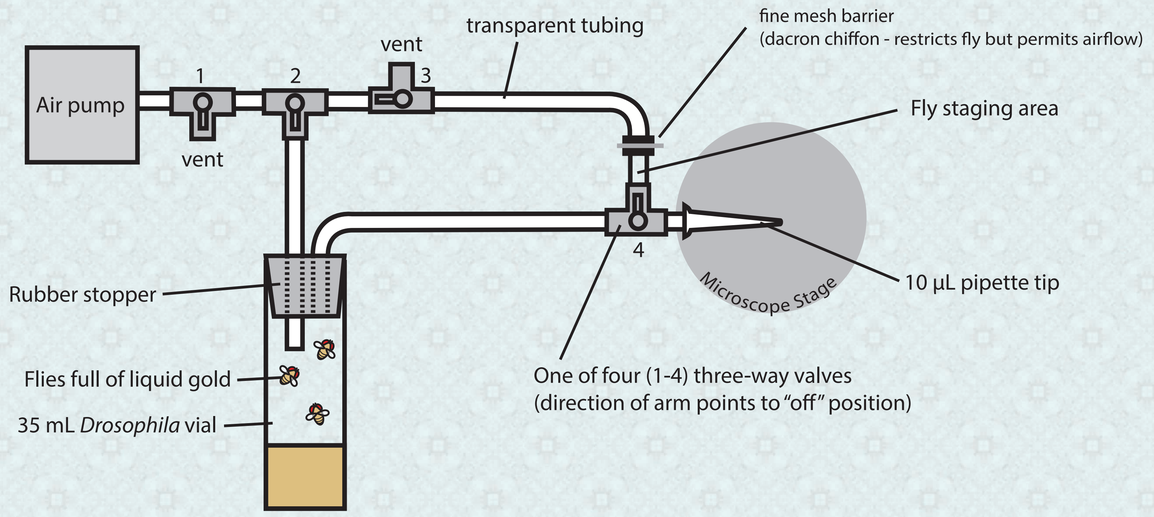
Concentrations of ions in hemolymph of male *D. melanogaster* (5d post-emergence), measured using ion-selective microelectrodes *in vitro*. *n*=15 (Na^+), 21 (K^+).

References & Acknowledgements

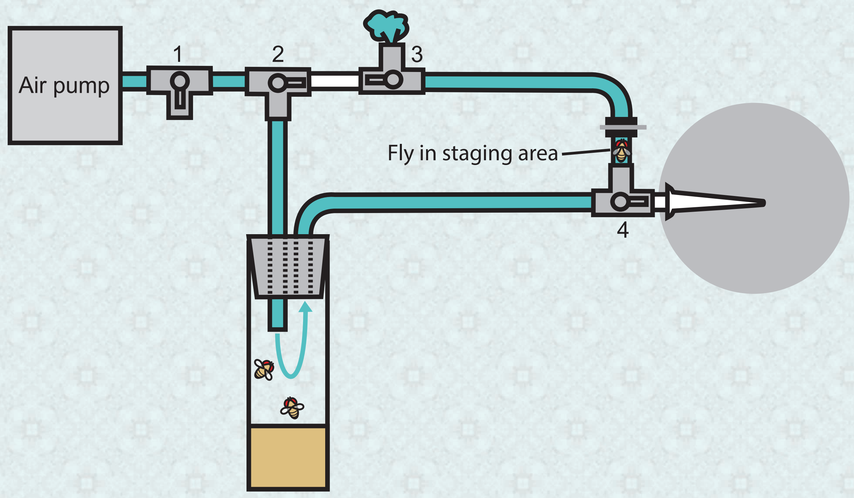
(1) Piyankarage, S.C., Featherstone, D.E., & Shippy, S.A. (2012) Anal. Chem. 84: 4460–4466. **(2)** Van der Meer, J.M., & Jaffe, L.F. (1983). Dev. Biol., 95, 249–252. **(3)** Haselton, A.T., & Fridell, Y.-W.C. (2011) JoVE 52: 1–5. **(4)** Naikkhwah, W., & O'Donnell, M.J. (2011). JEB 214: 3443-3354.

Thanks to the Sinclair lab for their useful comments and Andrew Donini for access to the ion-selective microelectrodes.

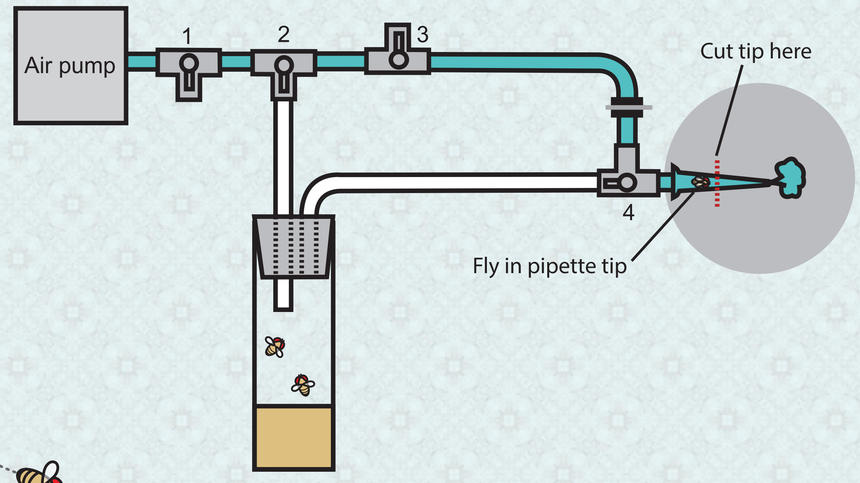
The hemolymph extractor



Hemolymph collection

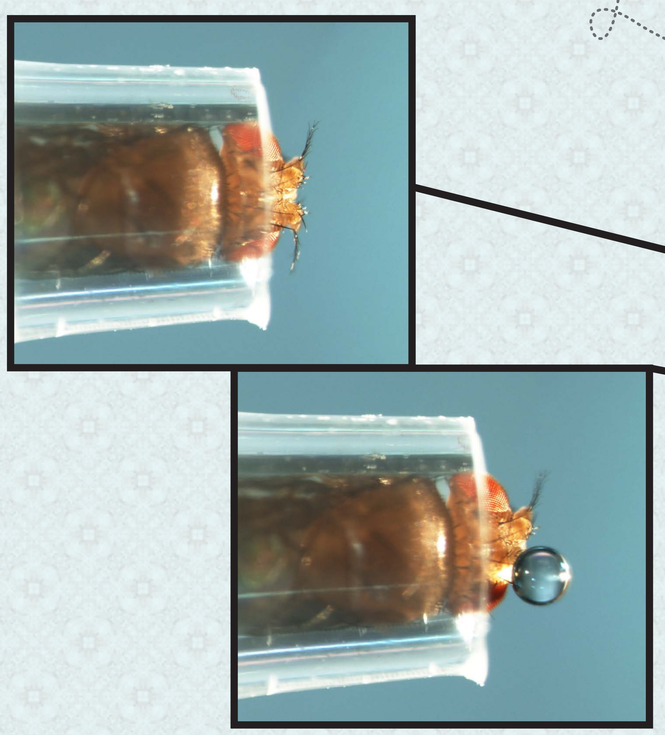


1 Airflow is directed through the fly vial, where a fly is picked up by the air stream and directed into the staging area.



2 The valves are adjusted, such that airflow pushes the fly from the staging area headfirst into the pipette tip.

3 Once the fly is restrained by the inner diameter of the tip, the excess tip is cut off with a razor, anterior to the fly.



4 Air pressure is applied until one or both antennae protrude from the pipette tip.

5 An antenna is amputated at the scape (basal segment) using forceps and a droplet of hemolymph emerges from the head of the fly (pressure can be applied to increase the volume obtained).

6 The droplet can be collected by microcapillary or placed under oil.

7 A fresh pipette tip is attached to the device and another fly can be sampled.