Table I. Probing frequency of female *Simulium venustum* as related to temperature

Temperature (°C)	No. of flies tested	No. of flies probing	Flies probing (%)
26.0	50	0	0.0
27.0	30	0	0.0
28.0	20	1	5.0
29.0	40	7	17.5
30.0	30	0	0.0
31.0	90	6	6.7
32.0	40	10	25.0
33.0	90	18	20.0
34.0	120	39	32.5
34.5	30	10	33.3
35.0	80	23	28.7
35.5	70	24	34.3
36.0	70	29	41.4
36.5	80	29	36.2
37.0	80	39	48.7
37.5	100	44	44.0
38.0	80	25	31.2
39.0	40	5	12.5
40.0	40	4	10.0

Table II. Chemicals tested as gorging stimulants for *Simulium venustum*

Test chemical	A[a]	B[a]	C[a]	D[a]	E[a]
10^{-4} *M* ATP	120	52	46	43.3	88.5
10^{-5} *M* ATP	120	50	8	41.7	16.0
10^{-4} *M* ADP	120	53	50	44.2	94.3
10^{-5} *M* ADP	120	56	26	46.7	46.4
10^{-3} *M* serine	40	16	1	40.0	6.3
10^{-3} *M* leucine	20	12	0	60.0	0.0
10^{-3} *M* alanine	20	9	0	45.0	0.0
10^{-3} *M* proline	40	13	1	32.5	7.7
Control	120	38	1	31.7	2.6

[a] Column A, sample size; column B, number of flies that probed; column C, number of flies that gorged; column D, percent that probed; column E, percent that probed that gorged.

location of the host using stimuli which operate over some distance, e.g., colour, carbon dioxide, and visual cues, c) landing and probing, and d) ingestion of blood. The flies with which we worked had already completed phases a) and b) and had landed, and were 'primed' to start probing.

Temperatures between 26 °C and 40 °C were tested to determine the effect of heat on probing. During these experiments a saline control or test compound (see below) was in the well of the feeding chamber. There was an overall increase in the percent of flies probing from 26 °C where none probed, to a maximum at 37 °C, where 48.7% probed (Table I). Between 37 °C and 40 °C a sharp decline in the probing rate occurred and, in fact, a couple of the flies died during the experiment, apparently due to excess heat.

During probing, the blood of a host is sampled for its suitability for ingestion. Because adenosine compounds having high energy phosphate bonds are known gorging stimulants in other haematophagous insects[5-7]; ATP and ADP were tested for their effects on gorging by simuliids. The amino acids, leucine, serine, alanine, and proline were also tested. Test compounds were dissolved in 0.15 *M* saline buffered to pH 7.2 with Sorensen's phosphate buffer. The buffered saline also served as a control. All experiments were conducted near 37 °C.

Table II shows that for female *S. venustum* 10^{-4} *M* and 10^{-5} *M* ATP and ADP elicit significantly more gorging than the control. At 10^{-4} *M*, ATP and ADP are equally effective as gorging stimulants. At 10^{-5} *M*, ADP elicits more gorging than ATP but both are less effective than at 10^{-4} *M*. Hosoi[7] reported that for *Culex pipiens* var. *pallens* ADP elicited gorging by more mosquitoes than ATP. Some spurious gorging occurred on the amino acids and the control, but the data are not statistically significant (Table II).

In addition to work on blood feeding mechanisms and associated factors, this technique may facilitate nutritional studies, eventual laboratory colonization of simuliids, and infection of simuliids with vertebrate pathogens without the need to feed on an infected host[8].

Résumé. On décrit une technique simple et pratique servant à nourrir des simuliidés à travers des membranes de latex commercialement disponible. On démontre avant tout que la chaleur est un facteur essentiel qui induit le sondage et que l'adénosine triphosphate et l'adénosine diphosphate stimulent l'engorgement de *Simulium venustum* Say.

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[5] W. G. Friend, Can. J. Zool. *43*, 125 (1965).
[6] R. Galun and J. Margalit, Nature, Lond. *222*, 583 (1969).
[7] T. Hosoi, J. Insect Physiol. *3*, 191 (1959).
[8] We thank the Medical Research Council (grant No. MA 2909) for financial support, the Ministry of Natural Resources for use of facilities at the Wildlife Research Station, Algonquin Park, Ontario, and Dr. W. Friend for use of the feeding chambers.

A Scanning Electron Microscope Method for the Examination of Glass Microelectrode Tips Either Before or After Use

High resolution methods for the electron microscopic examination of glass microelectrodes generally require that the tips of the electrodes be broken from the shank during mounting because of space limitations of the microscopes[1-5]. In the present study scanning transmission electron microscopy was used to view freshly drawn or previously used microelectrodes without damaging them. Operation of the scanning electron microscope in a transmission mode eliminated the need for coating.

[1] J. T. Alexander and W. L. Nastuk, Rev. scient. Instrum. *24*, 528 (1953).
[2] R. F. Bils and M. Lavallée, Experientia *20*, 231 (1964).
[3] D. S. Forman and W. L. R. Cruce, Electroenceph. clin. Neurophysiol. *33*, 427 (1972).
[4] K. Frank and M. C. Becker, in *Physical Techniques in Biological Research* (Ed. W. L. Nastuk; Academic Press, New York 1964), vol. 5A, p. 22.
[5] J. T. Tupper and H. Tedeschi, Proc. natn. Acad. Sci., USA *63*, 370 (1969).

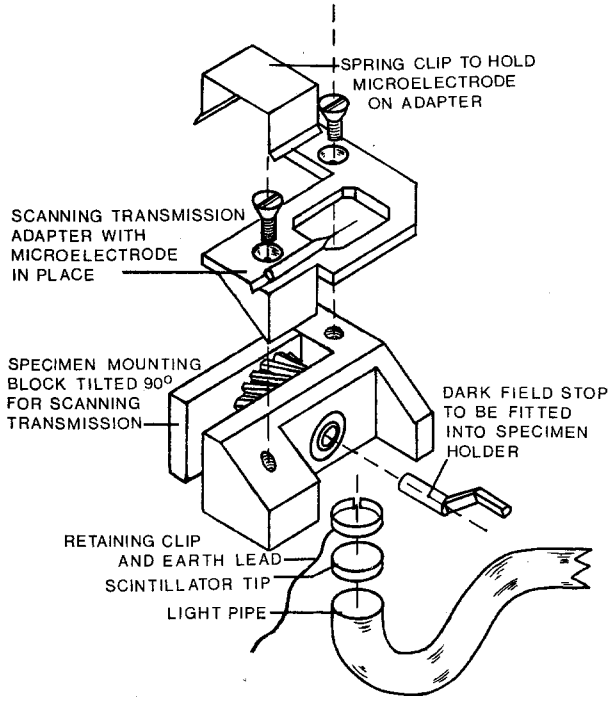

Fig. 1. Exploded view of a Cambridge Stereoscan specimen mounting block with modifications for use in a dark field scanning transmission mode.

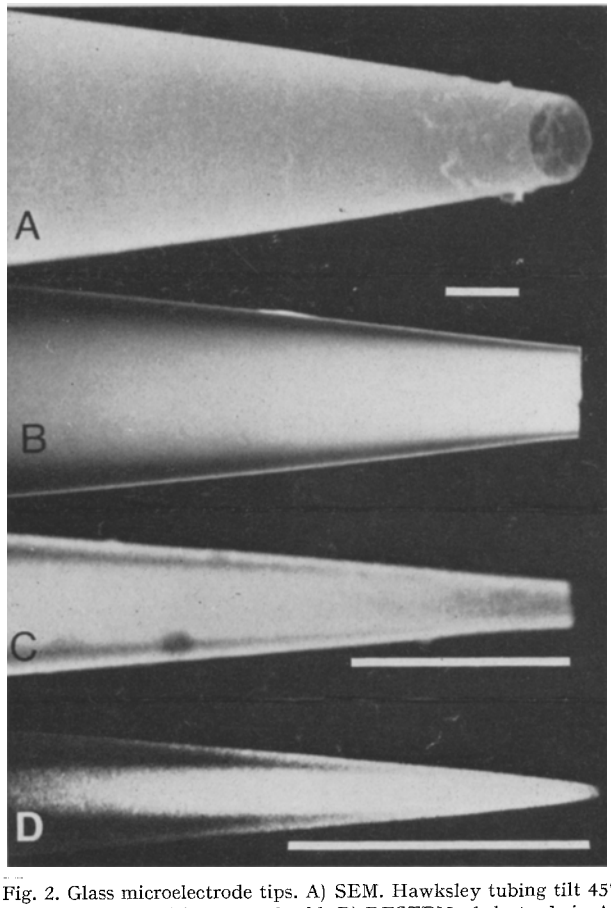

Fig. 2. Glass microelectrode tips. A) SEM. Hawksley tubing tilt 45° electrode coated with 10 nm of gold. B) DFSTEM of electrode in A. Wall thickness can be estimated closely at the tip. 10,000×, scale 1 μm. C) DFSTEM Yankee micropet. Filled and emptied electrode. Resistance before microscopy 12 $M\Omega$. Resistance after microscopy 28$M\Omega$ 30,000×, scale 1 μm. D) DFSTEM Yankee micropet (Low softening temperature) Tip of electrode is apparently sealed. 42,000×, scale 1 μm.

A Cambridge Stereoscan S4 Scanning Electron Microscope (Cambridge Scientific Instruments Ltd., Cambridge, England) with attachments for scanning transmission (STEM) was used with a modified beam blanking device for dark field transmission microscopy (DF STEM). Transmission microscopy is achieved by changing the position of the scintillator so that it is directly in the path of the electron beam. No electron collector is required. The dark field stop was a short length of $^1/_8$ inch stainless steel rod bent as shown in Figure 1 and inserted into the specimen holder so that it could be rotated in and out of the beam using the specimen rotation control.

Glass microelectrodes were pulled from 1.5 mm capillary tubing on a horizontal two-stage puller (Industrial Science Associates, Ridgewood, N.Y.). Glass of high softening temperature was: 1. Yankee Micropet 25 λ capillary pipettes (Clay-Adams, Parsippany, N.J.). Lower softening temperature glasses were 2. a different batch of Yankee Micropets, and 3. Hawksley microhaematocrit tubes (Hawksley & Sons Ltd., Lancing, Sussex, U.K.). Electrodes were filled by boiling in methanol under reduced pressure for 5 min, and then immersed in distilled water for 30 min. 3 M KCl solution was injected into the barrels, and at least 12 h allowed for equilibration. The external solution for measuring resistances was physiological saline. Electrode tips were examined either before filling or after measuring electrode resistance. Filled electrodes were emptied by flushing barrels with distilled water and immersing electrodes in water for 24–72 h to allow the filling solution to diffuse out of the tip before vacuum drying.

Dark field scanning transmission electron microscopy provided excellent high-resolution views of microelectrode tips (Figure 2) and allowed estimation of lumen diameters and tip taper angles. Glass of high softening temperature gave electrodes whose tips were open and whose included tip angle was 4–15°. The ratio of outside diameter: inside diameter at the tip was generally less than that of the original tubing in agreement with other reports[6]. Glass of relatively low softening temperature frequently produced electrodes whose tips were virtually closed, no orifice being resolvable at 40,000×. Such ultrafine electrodes are suitable for intracellular recording from small cells[7], some degree of tip breakage apparently occurring on first contact with the tissue. STEM did not damage electrodes as they could be filled normally and used for intracellular recording from cultured skeletal muscle cells. Electrodes which were filled to measure resistance and then emptied always had some internal salt crystalization. Refilling electrodes was generally not successful but electrodes which did refill had measured resistances in reasonable agreement with the initial filling (Figure 2).

Scanning electron microscopy in the secondary emission mode (SEM) provides sufficient resolution but requires coating the electrode with metal. The metal coating masks fine details of the electrode tip[8] and destroys the usefulness of the electrode for physiological recording. With moderately high accelerating voltages (30 kV) the spot current required for adequate secondary emission (about 1 nA) has a tendency to melt the thin glass tip. Spot current can be reduced but the decreased signal-to-noise ratio necessitates very long exposures for

[6] E. UJEC, Z. VÍT, F. VYSKOČIL and O. KRÁLÍK, Physiologia bohemoslov. *22*, 329 (1973).
[7] R. D. PURVES, G. E. MARK and G. BURNSTOCK, Pflügers Arch. ges. Physiol. *341*, 325 (1973).
[8] K. T. BROWN and D. G. FLEMING, Science *185*, 693 (1974).

adequate micrographs. Electrodes may be viewed uncoated at low accelerating voltages (less than 2 kV) but the resolution obtained is not sufficient to examine fine electrode tips.

The problems associated with secondary emission can be eliminated by using transmission microscopy. Samples do not need a metal coating and require much less spot current (about 1 pA). The transmission mode has the added advantage of greater attainable resolution than the secondary emission mode. The silhouette image obtained in the transmission mode can be improved by using dark field which allows determination of tip lumen diameter.

In summary, glass microelectrodes were examined with dark field scanning transmission electron microscopy without the necessity of heavy metal coating. The electrodes were not damaged and could be used for physiological recording after examination[9].

Zusammenfassung. Mikroglaselektroden wurden im rasterelektronenmikroskopischen Dunkelfeld transmissionsoptisch ohne Metallbeschichtung untersucht, wobei die Elektroden nicht beschädigt werden und für physiologische Ableitungen wieder benützt werden können.

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A Simple Method for the Microscopic Examination of Unsquashed, Stained Organs of Insects

In cytologic-microscopic examination of unsquashed objects of insects, with the dyes acetic-lacticorcein[1], carbolfuchsin (Fa. Merck), which are used for the staining of the nucleus, the difficulty arises, that either the object can only be examined in the upper or lower level based on the strong colouring, or the dye is not able to percolate completely by shorter duration of staining. To make possible an equal consideration of the nuclei at all levels, a method was evolved which proved useful for testes follicles of insects, and which is also suitable for discovering the metaphase-I-stages in oocytes.

The testicle is divided in Ringer's solution in separate follicles. The follicles are transported to a small dish with acetic-lacticorcein[1] and are left covered for at least 2 h at room temperature until the object is totally stained. Subsequently the follicle is transported to a slide, on which broken fragments of cover-glass are placed. The cover-glass fragments must be higher than the object to avoid squashing. After covering with a cover-glass, 60% acetic acid is added on one side, and on the other side the dye is sucked off with filter paper, until the liquid sucked off is nearly colourless. The cover-glass is sealed with cover-glass cement. According to the thickness of the object, the slide is kept on for 15 min to 2 h after the addition of the acetic acid. During this time the dye, which still remained in the cytoplasma of the follicle, becomes transparent and shows in all parts a uniform colouring.

[1] L. F. La Cour, Stain Techn. *16*, 169 (1941).

Part of a testicle follicle of *Oncopeltus fasciatus* (Heteroptera), 5th larval stage, from an unsquashed preparation. Acetic-lacticorcein. Note the nuclei situated in cysts.