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New Method for Relaxing Paraffin Sections in Histochemical Fluorescence Experiments

HISTOCHEMICAL fluorescence experiments, using the modifications[1,2] of the formaldehyde condensation procedure described by Falck *et al.*[3], are increasingly common. The procedures for demonstrating monoamines in freeze-dried tissue, require that the sections cut from formaldehyde gas-treated tissue be kept away from water, and so the conventional heated water bath for relaxing the cut paraffin sections cannot be used. Acetonitrile[4] and liquid paraffin[5] have been utilized and some laboratories even use a heated mercury bath (S. Norr, personal communication), although what is desired is a very non-reactive liquid. 'Fluorinert Electronic Liquids FC-75, FC-77' (3M Company, St Paul, Minnesota 55101) adequately replace the water bath. At 45° C these two clear liquids allow cut sections to be handled in the same manner as in the conventional water bath. Other liquids in this series can probably be chosen to give similar characteristics at other bath temperatures, although they have not been tried. A bath with intermediate qualities could be made as the liquids in the series are completely miscible with one another. The 'Fluorinert Electronic Liquids' are stated by their manufacturer to be very non-reactive and to have very low water and oil solubilities. The inertness of these liquids suggests that they may also be used to float sections as they are cut, and to immerse freeze-dried tissue for storage.

I thank 3M Company for the samples used and for information concerning their properties and S. Norr for bringing to my attention the use of heated mercury baths and the testing of 'Fluorinert Electronic Liquids' to relax paraffin sections for fluorescence studies.

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Some Responses of Tsetse Flies to Visual and Olfactory Stimuli

THERE is evidence that *Glossina morsitans* Westwood and *G. swynnertoni* Austen respond to moving objects from distances of 150–400 yards[1–3], and visual stimuli have been assumed to play the dominant part in host location by these "savanna" species. But it has been suggested that both species usually feed when the host animals are resting in relatively dense vegetation, where visual stimuli might be expected to be less important than olfactory stimuli[4,5]. There is some evidence that both species respond to host odour or that of dung in the field[1,6,7], but the role of olfactory stimuli in host-finding is not understood. I describe here experiments which demonstrate that there are differences between male and female *G. morsitans* in their response to visual stimuli, and that these responses are influenced by olfactory stimuli from a host animal.

Experiments were conducted in a wind tunnel which produced a near laminar airflow at 0.4 m s^{-1} in a large flight chamber. The flies were released from an open-ended metal tube 12.5 cm diameter and 45 cm long which protruded into a netting funnel opening into the flight chamber through the centre of the downwind end screen. An airstream containing olfactory stimuli could be released directly upwind of the tube, so that any fly on the inside of the netting funnel or in the release tube was subjected to the olfactory stimulus. A 30 cm square of matt black 'Perspex' mounted on horizontal runners could be driven across the upwind end of the flight chamber, passing immediately in front of the point of release of the odour airstream, and out of the chamber at each end of its traverse. The front end of the release tube was 2.5 m from the odour release point and the moving target.

Pupae of *Glossina morsitans morsitans* Westwood were obtained from the Tsetse Research Laboratory, Langford, and they and the adult flies were kept in a 12 h light : 12 h dark cycle at 25° C and 50–60% relative humidity. Teneral (unfed) flies were tested 46–60 h after emergence. Four to seven flies of one sex were placed in each tube and, after a pre-experimental procedure designed to minimize visual and mechanical disturbance, were subjected to the stimuli while at rest on the inside of the netting funnel or in the release tube (more than 90% were in the tube). They were free to fly out into the flight chamber. All tests were conducted between 30 min and 2 h before lights-off and with the wind tunnel airflow at 25°–26° C and 50–60% relative humidity.

The olfactory stimuli were derived from a 4–9 month old Ayrshire bullock. Air was pumped from the apex of a canopy suspended over the calf, through 5 m of nylon tubing into glass conditioning apparatus, and was released into the wind tunnel at 25°–26° C and 5 l. min^{-1} (the relative humidity varied with the temperature in the calf shed which was between 10°–25° C). The olfactory stimulus contained components from the calf's body surface, exhalant breath and excreta.

In all experiments flies were subjected to a visual stimulus consisting of one or two traverses of the visual target at the beginning of each of two successive minutes. The odour airstream was released for 2.5 min starting 30 s before the first appearance of the target. All insects flying into the flight chamber during these 2.5 min were counted.

In the first experiment (Fig. 1*a*) the target was driven across the flight chamber and back at 0.25 m s^{-1} each minute (giving an angular velocity of 4.8°–7.5° s^{-1} according to the position of the insect). In a control experiment 21% of males took off when exposed to the airstream from the shed when the calf was not present. The level of response was the same as that of both males and females when this airstream was turned off, and in subsequent controls no airstream was released. The olfactory stimuli from the calf increased the response of males to 47.4% ($P < 0.01$). The response of females remained unaffected by the presence of calf odour.

A second experiment, otherwise identical to the first (Fig. 1*b*),