between the mushroom bodies. After forming chiasmata, they separate from one another and sweep downwards postero-ventrally, and finally emerge out of the brain in the form of 2 fine nerves, the nervi corpori cardiaci (NCC). The NCC having the NSM enter the CC from their anterior end. 3 min after stimulation very little quantity of NSM is observed in the CNC, axonal tracts and NCC, but the axons running to the aorta from posterior end of the CC, stain intensely (Figure 3). The NSM does not release into the wall of aorta but directly into its lumen. The NSM is lacking from the entire CNS after 5 or 10 min of stimulation (Figure 4).

Electrical stimulation, either through optic lobes, median ocellus or directly on the pars intercerebralis does not show any difference; practically all have the same result.

The electrical stimulation on the brain demonstrates several interesting points worthy of discussion. The electrical stimulus arizing at any part of the brain causes release of the NSM from neurosecretory cells of the brain. It thus confirms the observations of earlier workers that the release of NSM from the CNC is under the control of nervous electrical stimuli.

In most of the insects, the CC are main neurohaemal organs[12–14], while in some Hemiptera[15–18] only the aorta functions as a neurohaemal organ. In these insects, the axons of cerebral NSC terminate not in the CC but in the aorta wall directly and NSM stores in the aorta wall. On the other hand, in some orthopteroid insects[19] and *Calliphora*[20], the aorta functions as a secondary neurohaemal organ. In the present study, it has been observed that some axons from the CC pass into the aorta and there is no discharge of NSM in its wall, but it is directly discharged into the lumen of the aorta. Thus, the aorta here serves as only a releasing site for the cerebral NSM and not as a storage organ, and therefore the function of the aorta in the last instar nymph of *Orthetrum chrysis* differs from that of other insects.

Résumé. L'effet de la stimulation électrique sur le système neurosécrétoire cérébral de la larve d'*Orthetrum chrysis* révèle que la neurosécrétion cérébrale est emmagasinée dans les corps cardiaques et qu'elle est déchargée directement dans l'aorte.

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PRO EXPERIMENTIS

Artificial Feeding of Simuliids (*Simulium venustum*): Factors Associated with Probing and Gorging

Simuliids (Diptera, Simuliidae) are of consequence to human welfare as noxious pests and as vectors of human and animal disease agents, notably the blinding filarial worm, *Onchocerca volvulus*. Despite their importance, knowledge of the mechanisms and factors associated with blood feeding in simuliids is wanting. Other workers have fed simuliids on cotton balls soaked with dextrose and defibrinated blood[1] and on whole blood through skins of young rats and chicks[2,3]. These procedures, however, do not lend themselves to studies with chemically defined media and the skin membranes are difficult to prepare. Herein, we describe a simple and convenient technique for feeding simuliids through inexpensive, commercially available, latex membranes and present preliminary evidence that heat is an essential factor in inducing probing and that adenosine triphosphate and adenosine diphosphate are gorging stimulants.

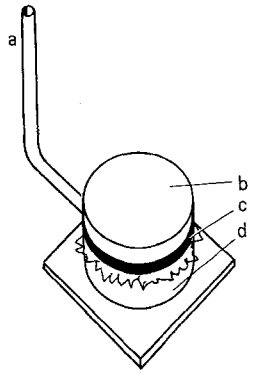

Diagram of artificial feeding chamber. a) right angle glass tube; b) membrane surface; c) rubber ring for attachment of membrane; d) side of plexiglass well.

The feeding chambers used are a modification of those developed by FRIEND and CARTWRIGHT[4] for feeding *Rhodnius prolixus*. Each chamber consists of a plexiglass well with an attached right angle glass tube through which test solutions are introduced (Figure). A latex membrane (Sheik® regular prophylactics) is fitted over the well and secured by a rubber ring. The chambers are heated on a slide warmer. Thermocouples are inserted into the well through the glass tube and the temperature of the test solution monitored with a Leeds and Northrup® temperature potentiometer.

In our experiments female *Simulium venustum* Say were caught individually in vials as they landed on a human subject. Each vial was inverted on the membrane for 5 min during which time the flies were observed as to whether they probed and gorged. Studies were conducted at the Wildlife Research Station, Algonquin Park, Ontario, Canada. Significance of results was determined using the *Z*-test.

The response of simuliids to hosts can be divided into 4 general phases a) activation, b) orientation to and

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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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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.

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