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# THE STUDY OF INSECT BLOOD-FEEDING BEHAVIOUR. 2. RECORDING TECHNIQUES AND THE USE OF FLOW CHARTS

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*This paper continues a discussion of approaches and methodologies we have used in our studies of feeding in haematophagous insects. Described are techniques for directly monitoring behaviour: electrical recording of feeding behaviour via resistance changes in the food canal, optical methods for monitoring mouthpart activity, and a computer technique for behavioural event recording. Also described is the use of "flow charts" or "decision diagrams" to model interrelated sequences of behaviours.*

*Electrical Recording*

In large insects such as *Rhodnius prolixus* and *Triatoma infestans*, a short length of wire can easily be implanted in the haemolymph of the thorax, and connected to recording circuit (Fig. 1). If a second piece of wire is inserted into the diet and also connected to the recording circuit, an electrical signal can be derived which can be correlated with various activities associated with feeding.

This technique is an adaptation of that used in studies on aphids (McLean & Kinsey, 1964; Tjallingii, 1985); with such small insects, the electrode is merely affixed to the dorsal cuticle rather than inserted through to the haemolymph. In studies on the mosquito, electrical connection with the inside of the insect depended on contact between the insect's feet and a metal grid on which it stood (Kashin & Wakeley, 1965). In early experiments with *R. prolixus*, such methods produced a noisier signal, probably due to the extra and sometimes variable resistance introduced by the insect's cuticle being interposed between electrode and haemolymph. Nevertheless, this simpler technique can be used to monitor penetration, for example, or to determine when pumping commences.

The technique has been described in some detail in Smith & Friend (1970). Visual observations (see next section) made during recordings have enabled the several distinct components of a typical recording (Fig. 2) to be identified with initial penetration, maxillary probing, tasting or sampling, and operation of the pharyngeal pump. We were also able to determine that, in the absence of gorging stimulants such as adenosine triphosphate (ATP), the probing phase was prolonged and the pumping phase did not start (Friend & Smith, 1971). In later work (Smith,

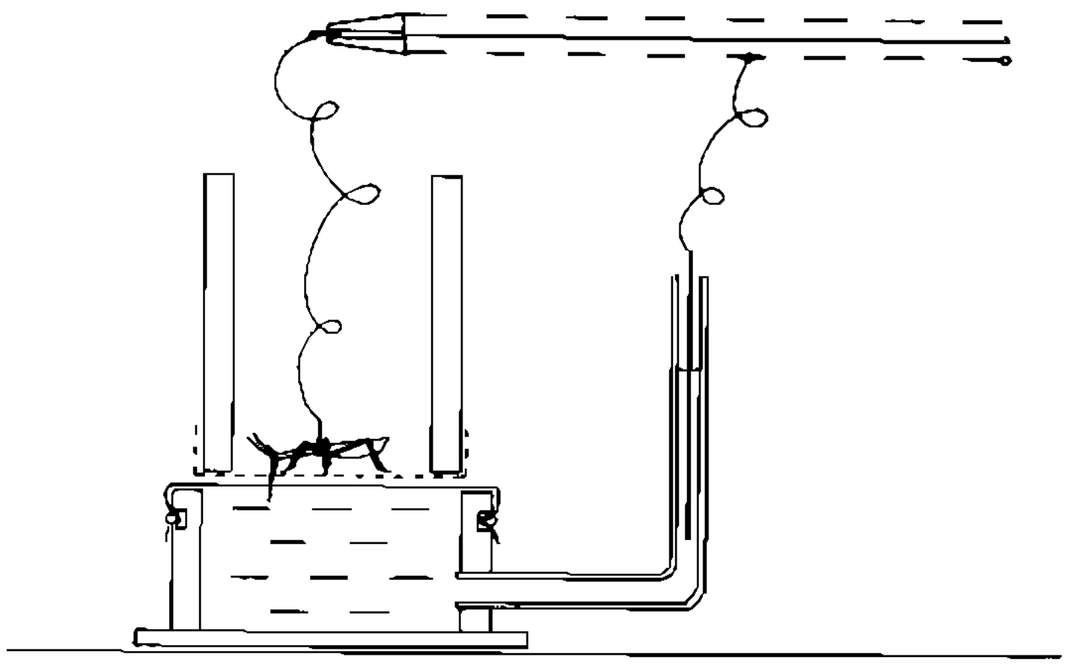

Fig. 1: Apparatus for electrically monitoring blood-feeding in *Rhodnius prolixus*. A short length of platinum wire in a side-arm of the diet chamber is the second electrode, connected to the grounded shield of a coaxial cable and connected in turn to a preamplifier or high gain oscilloscope. Connection can also be made to the insect by attaching the active electrode lead to the metal mesh floor of the insect chamber (dotted). Modified from Smith & Friend, 1970.

1979), this technique was used to study the action and control of the pharyngeal pump, since the records have sufficient resolution to allow individual strokes of the pump to be counted and their frequency determined. A simpler circuit was used in this work; serendipitously, we discovered that no voltage source was needed to produce an adequate signal, and the electrodes in insect and diet could be connected directly to a standard high input-impedance micro-electrode preamplifier, such as a Grass P-15 (to see the AC component of the signal), or to a DC amplifier on an oscilloscope.

Although the origin of the signal current in this latter case is unknown, the varying signal probably reflects changes in the resistance to electrical current of the food canal as, for instance, the pump opens and closes or the

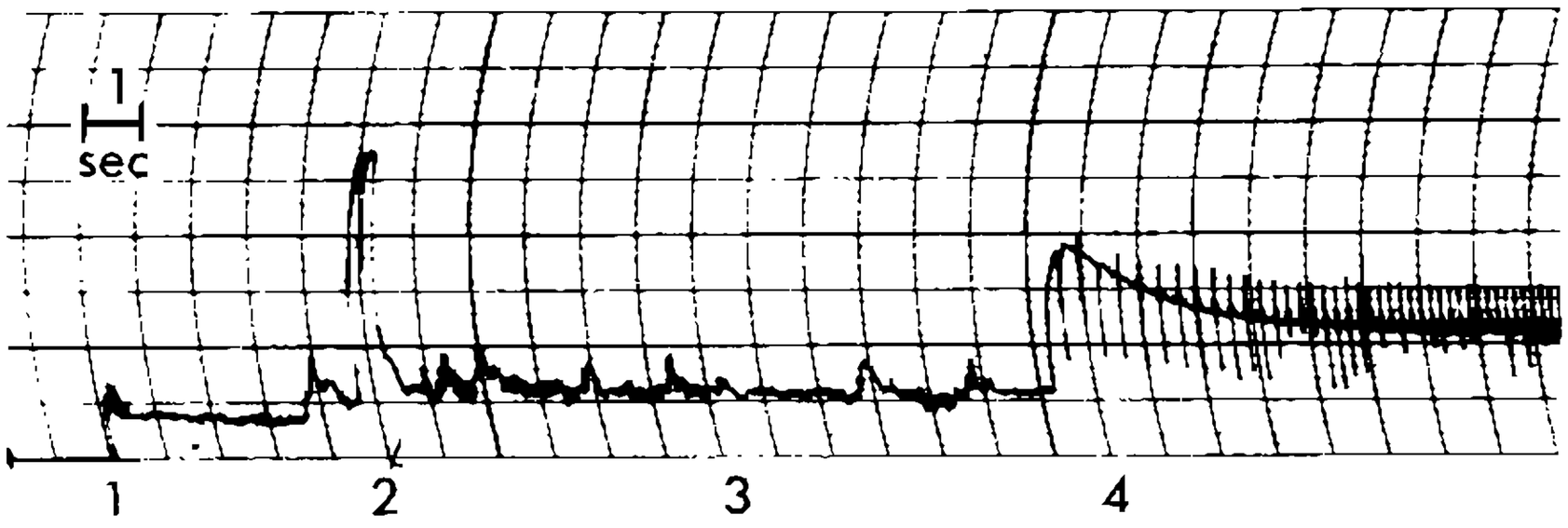


Fig. 2: Typical record of electrical activity associated with *R. prolixus* feeding on saline and ATP, and using apparatus shown in Fig. 1. Vertical axis represents voltage at preamplifier input (arbitrary units).
1. Initial penetration, 2. "Sampling" – opening of functional mouth with one pump stroke (negative-going "spike"); 3. Maxillary probing, with somewhat irregular 1-3 second periodicity correlated with protraction-retraction cycles; 4. Start of pumping phase, with individual pump strokes showing increase in pump frequency from 2 $s^{-1}$ to 6 $s^{-1}$. Modified from Smith & Friend, 1970.

functional mouth opens. Alternatively, it may reflect changes in velocity of the fluid column in the food canal.

*Visual Monitoring*

To correlate the electrical events recorded during feeding with the activity of the mouthparts, we developed methods for filming live insects probing into artificial diets (Friend & Smith, 1971). Special chambers (Fig. 3) were made from glass microscope slides held apart by glass tubing; a narrow membrane of prophylactic rubber was glued to the edges of the slides. Confined to a small feeding chamber, *R. prolixus* probed into the warmed diet through a restricted area of the membrane. In this way, the mouthparts could be quickly located under low power of a compound microscope and filmed with either low (2.5X) or medium (10X) power objectives. To visualize movement of the diet into the functional mouth as the insect pumped, we used a dilute suspension of frog red blood cells; these were large enough to be seen easily under medium or even low power, particularly when phase contrast or dark field optics were employed. Phase contrast also clearly revealed the secretion of saliva during the maxillary probing phase, and occasional momentary regurgitation of diet mixed with saliva.

The electrical and visual recordings were combined into a single television image to de-

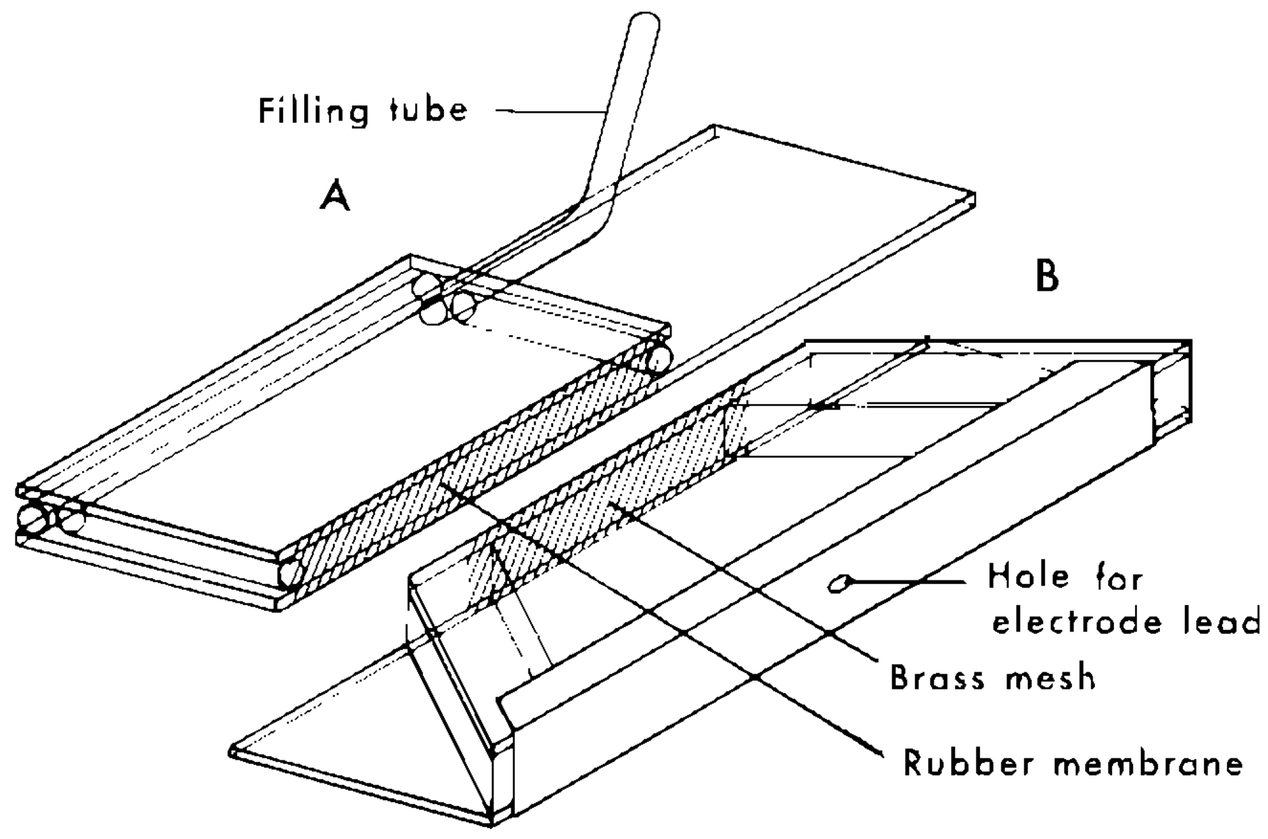


Fig. 3: Apparatus for visual observation of mouthpart activity in feeding *R. prolixus*. The insect is confined in chamber B, and the diet is injected into chamber A by filling tube. The diet is warmed by focussing a microscope lamp (without heat filter) onto a piece of black tape (not shown) on the upper surface of chamber B. When the chambers are brought together, the insect probes through the brass mesh onto rubber membrane, and inserts its maxillae into the diet chamber. Here they may be observed microscopically through the slide comprising the chamber's upper surface. Modified from Smith & Friend, 1971.

termine the exact correlation between the two sets of events (Smith & Friend, 1971). We used a simple video mixer and two television cameras, each of which had a portion of its field of view obscured. One camera was affixed to the microscope, and the other was focussed on an oscilloscope screen displaying the electrical signal. Using slow motion and single frames on playback through a video tape-recorder, we could determine for instance that each thrust and withdrawal of the maxillae was accompanied by low amplitude periodic changes in the electrical signal, and that a smaller and higher frequency signal superimposed on each thrusting cycle corresponded to characteristic "flicking" motions of the maxillae during protraction and retraction.

*Event recording by computer*

A common technique in analysing behaviour is to record the times of occurrence of a number of different specific actions, so that counts of each and their relative frequencies and timings may be later analysed statistically. Many different devices have been developed to assist in this task, which must often be done in real time in the field. We have found that a useful approach is to program a portable battery-powered computer to record the time when any one of several designated keys is pressed. We used a Tandy-Radio Shack Model 100 computer for the task, although other computers might be equally suitable. A simple Basic program allows the experimenter to choose which keys will represent which actions, and what names to give them. Upon pressing the start key, the program reads the computer's internal clock to establish time zero. Then whenever a designated key is pressed, the timer is reread, and the value entered into an array. One key allows the most recent key press to be cancelled, its time being erased from the array. When the stop key is pressed, the program displays various options including immediate analysis, storage in a file, and erasure. For analysis, such computations as total time spent in each activity and the number of times each activity occured can easily be made and displayed on the screen or sent to a printer. Further analysis can be done either by addtional programs in the portable computer, or by uploading the data to other computers.

We have used such a system for studying host examination behaviour prior to oviposition in the parasitoid wasp *Trichogramma minutum* (Schmidt & Smith, 1987). Further details of the program may be obtained from the authors.

*Behavioural decision diagrams*

For conceptualising what is know about a particular behaviour, "flow charts" or "decision diagrams" are useful tools. Perhaps even more valuable, the exercise of putting down on paper the various actions in a behaviour, how they relate, and what information is used to elicit them, reveals what is *not* known, or what is conjectural, as well as what is known.

Fig. 4 is such a chart, constructed for feeding behaviour in *R. prolixus*. Each action is indicated by a rectangular box, each decision point by a diamond, and end points by rounded rectangles. Directional arrows link the boxes, such that the behaviour under particular conditions is described by following the indicated path, and making the appropriate choice at each diamond.

The scheme showed in Fig. 4 is tentative. Some parts, such as the responses to ATP, are based on published evidence. Other parts are attempts to conceptualise observations that still need to be tested experimentally and quantitatively. The whole diagram is presented here for illustrative purposes, and perhaps provocatively to suggest areas of research. However, we will not attempt to describe or justify most of the diagram in this paper.

In Fig. 4, *R. prolixus* is assumed to start at rest, in its typical "dormant" state (the "akinesis" of Wigglesworth & Gillett, 1934). In the laboratory, such an insect "wakes" when presented with a variety of stimuli (turning on the lights, movement, breathing into the container, etc.). The first decision depends on the internal state of the insect: we use the term "hunger" simply to mean that the insect is "hungry" if its behavioural actions are consistent with those culminating in taking a blood meal. Two factors which may influence the decision to move toward and investigate a potential host are its present degree of abdominal extension, and the time since it last fed. Insects which have gorged display neither host-seeking behaviour nor probing on warm objects when tested soon after feeding; since cessation of feeding appears to depend on information from abdominal stretch receptors (Maddrell, 1963), it seems reasonable that the same input changes the response to signals indicating a host. Nymphs fed a non-nutritive diet (saline and ATP) will excrete the meal rapidly; they will resume attempts to feed when about 66% of the meal volume has been excreted (Smith, J.J.B., unpublished observations). Although abdominal ex-

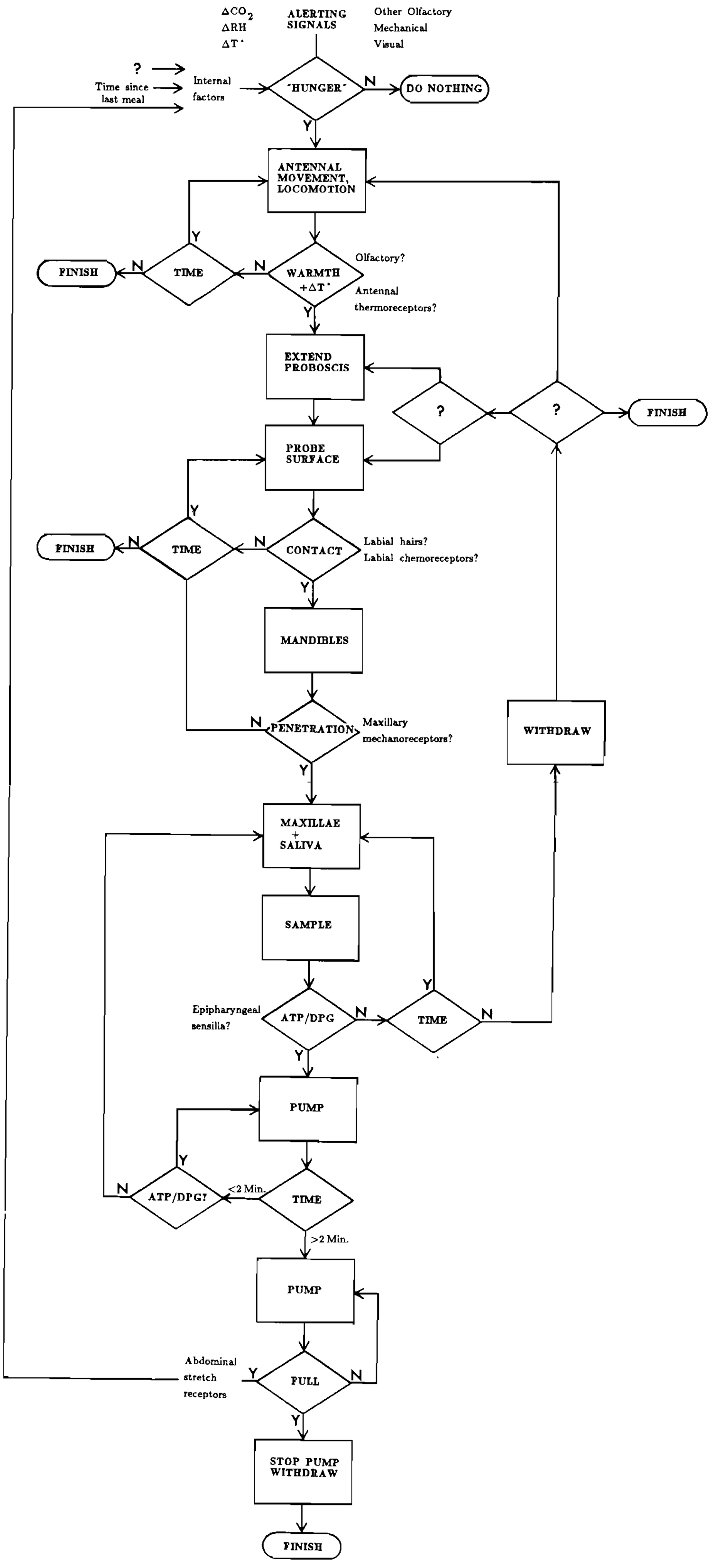


Fig. 4: Flow chart (or "decision diagram") of feeding behaviour in *R. prolixus*. For explanation, see text.

tension is a function of time, since the insect excretes much of the fluid component of the meal, the relation between these factors and "hunger" is complex. For example, an insect interrupted before it "voluntarily" ceases feeding will resume feeding if allowed to shortly afterwards. However, if the insect has taken sufficient blood to induce moulting, it will no longer feed when exposed to a host after about three days have elapsed. Such preliminary observations are currently under study in this laboratory.

The third input to the "hunger" decision, indicated by the "?", represents unknown factors – there are, for example, always those insects which will not feed even though they *should* be hungry.

One other component of Fig. 4 warrants mention. Several decision points are labelled "time". The earlier three represent the observation that the insect will persist for some time in its present behaviour despite absence of signals triggering the next behavioural act; however, it eventually "gives up" and either ceases to exhibit feeding behaviour at all, or returns to an earlier phase. The "time" decision following the decision to activate the pharyngeal pump embodies our preliminary observations that the phagostimulant ATP only needs to be present in the diet for about the first two minutes of feeding; if ATP is removed by exchanging the diet after about 2 minutes, the insect usually continues to feed on a saline diet to repletion (Friend & Smith, 1977).

Much work remains to be done to clarify the feeding behaviour of *Rhodnius prolixus*. A diagram such as shown in Fig. 4 represents a useful "snapshot" of present thinking; however, we fully expect substantial revisions, and increased complexity, as more is discovered.

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