A new method for obtaining blood from a small mammal without injuring the animal: use of Triatomid bugs

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Summary. By means of hungry Triatomid bugs, samples of blood (40–200 µl) can be obtained from small mammals, such as bats, without causing any harm to the animals. The blood can be extracted from the gaster of the bug immediately after the bug has finished sucking. The method was successfully used for measuring the energy budget by the doubly labeled water method, and for obtaining material for lymphocyte cultures, and may prove useful in other fields, too.
Key words. Blood sampling; Triatomidae; small mammals; lymphocyte culture; doubly labeled water method.

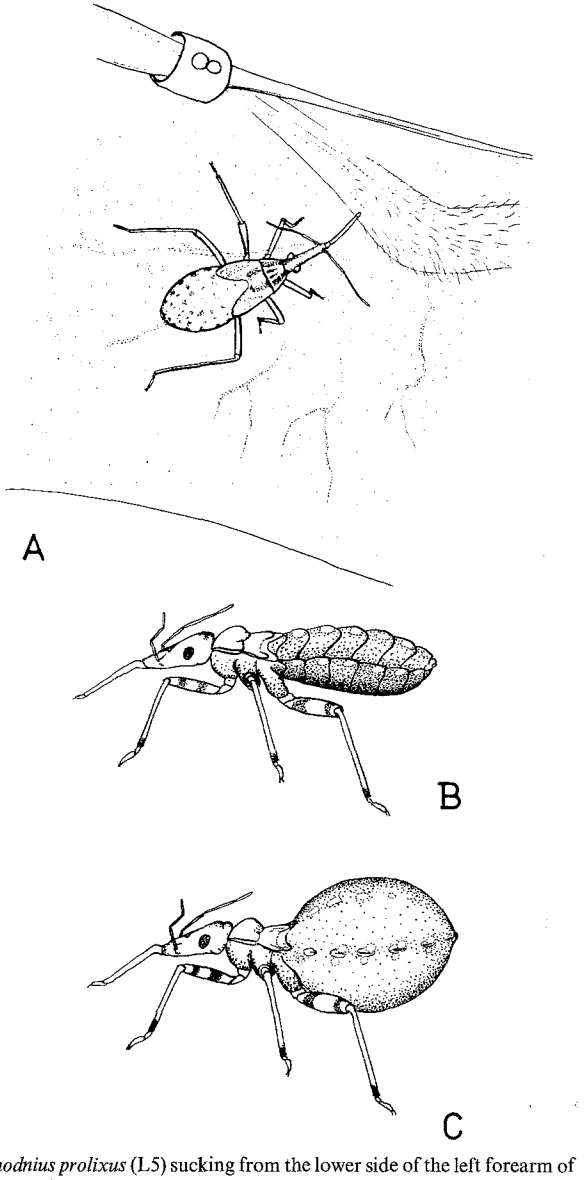
Sometimes it is necessary to take samples of blood from small mammals in a way that avoids not only injury but also agitation of the animal. Undisturbed behavior may be a prerequisite of the experiment, for example when measuring the energy expenditure or monitoring hormone titers of individual animals in their natural environment as well as under laboratory conditions. For this reason, we have developed a method allowing us to bleed a bat or another small animal in a way that causes little distress compared to the methods previously described[1–9] and requires no anesthesia, so that peripheral vasoconstricter response is avoided. We used blood sucking Triatomid bugs to get small amounts of blood from unanesthetized bats. The method has a parallel in plant physiology: Kennedy and Mittler[10] as early as 1953 described extraction of phloem sap using the mouth parts of aphids.

Description of procedure. According to the quantity of blood required, Triatomid bug species of different sizes or at different larval stages can be used. So far, we have used *Rhodnius prolixus, Triatoma infestans* and *Dipetalogaster maximus*[11]. To avoid contamination of the blood by bug hemolymph and excrements of the last meal remaining in the intestines, one should allow as much time as possible to pass (6 weeks up to several months) after the last blood intake (or the last molting that normally follows a sufficient blood meal). The bugs then look completely dehydrated and display a paper-thin abdomen (fig. B).

The bat was held gently in one hand and one wing unfolded with the other hand. Then a bug of the appropriate size was put onto the lower side of the wing near the elbow joint by a second person. The bug recognizes its victims by their higher temperatures compared to the surroundings. Therefore it is helpful to warm the wing membrane from the outside (e.g. by placing the warm finger tips behind it), when the body temperature of the bat is very low (e.g. on account of day sleep lethargy). The bug extends its proboscis, looks for a suitable place to bite – sometimes making several attempts to find a larger capillary – and begins to suck (fig. A). Within a very short time (sucking duration max. 4–8 min), the abdomen of the bug inflates like a balloon (fig. C). The bug bite is usually not noticed by the host animal. After the bug has retracted its proboscis, no wound whatsoever remains. When it is sufficiently filled, the bug can be removed with forceps before it stops sucking of its own accord. One can also wait for the sucking procedure to end: the bug then suddenly retracts its proboscis and immediately tries to escape into a dark hiding-place, which some bugs turn out to be amazingly clever at doing.

To obtain the blood, the bug was decapitated immediately after sucking, the lower side of the abdomen was disinfected with alcohol, and by means of a glass capillary or heparinized syringe the blood was extracted directly from the pricked gaster.

From L 5 of *Rhodnius* or L 4 of *Triatoma infestans* we obtained samples of circa 50–70 µl blood, with larger bugs (e.g. *Dipetalogaster maximus*) 0.2 ml and even more can be obtained. It is advisable, however, not to take more blood from a small mammal than circa 1% of its body weight at any one time. According to Rauch and Beatty[12] the total blood quantity of *Eptesicus fuscus* (Vespertilionidae) is about 7% of its total weight. 1% of its weight thus corresponds to as much as circa 14% of its blood volume. A blood extraction of about this quantity proved to be by no means harmful to the animals in our experiments.



A Rhodnius prolixus (L5) sucking from the lower side of the left forearm of a Glossophagine bat; *B* and *C: Triatoma infestans* (L4) starts (B) and finishes (C) sucking.

Most of the living blood cells are not damaged as digestion ferments are added only in the intestines of the bug[13]. Possible alteration of blood composition, i.e. loss of water and salts from the stomach during the sucking period, or possibly even more

drastic effects, such as the addition of substances causing crystallization of hemoglobin[14,15], as observed some days after a blood meal, should be taken into account and investigated, according to the requirements of the experiment.

Mixture of the blood with bug hemolymph while it is being extracted from the bug is conceivable. To estimate the maximum contamination with bug hemolymph, we squeezed out some 'empty' bugs that had not yet sucked and absorbed the hemolymph with filter paper: The maximum weight loss of the bug body weighing circa 20 mg amounted to 2 mg. A blood sample of 50 µl blood could therefore contain a maximum of 4% bug hemolymph; this estimate, however, is probably much too high, since strong squeezing during the blood extraction from the gaster of the bug can be avoided.

Applications. 1) Measuring the energy budget by means of doubly labeled water. For this method[16], blood samples have to be taken twice or several times from the experimental animals to allow measurement of the decrease of labeled hydrogen and oxygen in the blood. In experiments on flower-visiting bats[17] the blood extraction by means of Triatomidae yielded excellent results. Since it is only the ratio of the concentrations of labeled atoms which have to be evaluated for measuring the energy budget, neither a possible water loss during sucking time nor a slight but identical dilution of the blood through bug hemolymph or saliva would influence the results with this method.

2) Lymphocyte culture for karyological analyses: The blood obtained using bugs is suitable for lymphocyte cultures. We diluted a Heparin solution[18] up to a concentration of circa 1400 IU/ml with balanced salt solution. A small quantity of it (~ 50 µl) was taken in the syringe prepared for extracting the blood from the bug. After addition of culture media a dilution of about 50 IU/ml was reached. The syringe was rinsed with medium or fetal calf serum to obtain all the blood. So far, the lymphocytes of 13 bat species have been successfully cultivated by this method[19].

3) The method may well be suitable for other purposes, even though possible changes of blood composition caused by the bugs have to be tested in each case. This method may also be applicable for measuring hormone titers, for immunological and further biochemical analyses, e.g. allozyme-analyses and paternity tests, which have become increasingly important over the last few years[20–22].

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Shortening velocity of single muscle cells isolated from a molluscan smooth muscle

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Summary. The maximal unloaded shortening velocity (V_{max}) of smooth muscle cells isolated from the pedal retractor muscle of *Mytilus* was more than twice as large as that of the whole muscle, suggesting the presence of extracellular components which resist the contraction of the whole muscle. The V_{max} of the isolated cells was almost constant at cell lengths ranging between 0.5 and 0.83 l_0 (l_0, optimal length for tension generation) indicating that the intracellular resistance to contraction is negligible within this range of lengths.

Key words. Molluscan smooth muscle; single smooth muscle cell; unloaded contraction; shortening velocity; *Mytilus.*

The mechanical properties of smooth muscle do not necessarily reflect those of individual muscle cells because of the small size of the muscle cells, which are often arranged in a complex geometry, and the abundance of connective tissue in the whole muscle. Therefore, in deducing the mechanical characteristics of the contractile elements from those of the whole muscle, it is important to consider the mechanical arrangement of the muscle cells and the effect of connective tissue. Studies made on several smooth muscles to deduce the mechanical properties of individual cells from those of whole muscles have suggested practically linear relationships between the mechanical properties of the cells and those of the whole muscles[1–4]. In these studies, the lengths of the cells during contraction or other mechanical events were estimated by direct microscopic observation of the muscle or by measurements of the lengths of the cells dispersed from the muscle after chemical fixation. However, the best way to study the mechanical properties of individual cells is to measure directly these properties in isolated, live smooth muscle cells[5,6].

In the pedal retractor muscle (PRM) of a bivalve mollusc, *Mytilus edulis,* the length-tension relations have been examined in both whole muscles[7] and isolated cells[6]. The length-tension relation of the isolated cells have shown that the range of lengths over which active tension is developed was much wider in the isolated cells (0.17 to more than 2 l_0, where l_0 is the optimal length for tension generation) than in the whole muscle (0.35 to 1.8 l_0). Such a difference in the 'working range' would be accounted for by assuming that the cells are connected in series by long intercellular linkages. The effective length of such linkages would correspond to about 0.3 l_0. If such linkages exist, the