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Demonstration of isobutyric acid in some triatomine bugs

Short communication

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Like most of the Reduviidae, adult stages of Triatominae possess two pairs of metathoracic scent glands. The ventrally situated metasternal glands open just anterior to the metacoxal cavities, and the dorsally situated Brindleys glands open just within the upper posterior angle of the metathoracic epimeron (Brindley, 1930; Carayon, 1971). The morphology and ultrastructure of both pairs of glands in adult *Panstrongylus megistus* (Burmeister) have been described by Schofield and Upton (1978). Immature stages of Triatominae possess neither metasternal nor Brindleys glands.

The secretions of the metasternal glands of adult Triatominae have not yet been identified. However, the Brindleys glands of *Rhodnius prolixus* (Stahl), *P. megistus*, and *Triatoma phyllosoma* (Burmeister) have been shown to secrete isobutyric acid, without other detectable components (Games et al., 1974; Pattenden and Staddon, 1972; Kälén and Barrett, 1975).

Isobutyric acid is both corrosive and pungent, and its ejection from the Brindleys glands can be observed under the dissection microscope when an immobilized adult triatomine bug (e.g. *P. megistus*) is vigorously prodded. A possible interpretation of these observations is that isobutyric acid is secreted for purposes of defense. It was of interest therefore, to see if this acid was secreted by species other than the three mentioned above.

The insects used were taken from colonies maintained for several years at the London School of Hygiene and Tropical Medicine in insectaries at 27° C and 40–80% RH. 20–25 adults (mixed males and females) of each of six species of Triatominae (*P. megistus* Burmeister; *R. prolixus* Stahl; *T. infestans* Klug; *T. maculata* Erichson; *T. brasiliensis* Neiva; and *T. vitticeps* Stahl), starved for 2 weeks, were placed in a clean glass vessel containing a magnetic stirrer. As the bugs were agitated by the slowly-revolving stirrer, a continuous, charcoal-filtered, water-saturated, airstream was passed over the bugs and then over a glass

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'finger' which was cooled by being filled with liquid nitrogen. The airstream was very slow, about 4 ml per min, and was passed by a suction pump placed after the glass finger to avoid contamination by the pump itself.

After 4–5 h, the ice which formed on the outside of the glass finger was washed off with about 10 ml of redistilled acetone, which was then dried by standing over anhydrous sodium sulphite powder for 24 h. The dried acetone was filtered under pressure through sintered glass and concentrated by rotary evaporation at 25° C to about 0.5 ml. 1 μ l samples of this were injected into a Perkin-Elmer F11 GLC onto a column of 2.5% silicone gum-rubber on Chromosorb-G at 180° C.

At least one sample of secretion from each species of bug revealed a small peak on analysis, with a retention time similar to authentic samples of isobutyric acid. These samples were then checked by simultaneous injection of sample plus an equivalent amount of 0.01% synthetic isobutyric acid in acetone, whereupon only one peak (of increased size) was apparent. No peaks other than that of isobutyric acid were seen.

In one experiment, a sample was derived from a group of *P. megistus* which had their Brindleys gland ostioles blocked with nail-varnish. This sample did not reveal a peak on analysis.

In the original analyses by Pattenden and Staddon (1972) and Games et al. (1974), the Brindleys glands were dissected out of individual bugs, dried, punctured, and the contents taken by micropipette for analysis by both temperature-programmed gas-chromatography and by mass-spectrophotometry. The simple analyses reported here are not conclusive, but are in agreement with the work of these authors, and with Kälén and Barrett (1975) who showed that air above 20 'scent-releasing' adult *R. prolixus* contained isobutyric acid. Thus, it is justified to assume that there are at least 7 species of Triatominae, representing 3 genera, which secrete isobutyric acid when disturbed. It is worth noting in addition that 10 other species of Triatominae, including *Dipetalogaster maximus* (in spite of a report to the contrary by Mazzotti, 1970), and some predatory reduviids of the genera *Reduvius*, *Zelus*, and *Zelurus* have released an odour when handled by the author, which seems similar to that of isobutyric acid. It is possible that isobutyric acid may be a characteristic secretion, not only of Triatominae as suggested by Games et al. (1974), but also of other Reduviidae which possess Brindleys glands.

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