ECV relative to changes in the intracellular volume (ICV) is consistent with the large increase observed in the R wave component of the ECG. This idea rests in the observation that the R wave component of the ECG reflects changes in the total longitudinal current in cardiac tissue. Since the extracellular component of the total current depends not only on the ratio of the external and internal specific resistances (R_o and R_i), but also on the ratio of the cross-sections of these two compartments[11,12], a change in these values will cause a change in the R wave amplitude. Here the 3-4-fold increase observed in the R wave component of the ECG of hibernating hamster is in good agreement with the 3-4-fold decrease in ECV observed in the ventricles of these animals.

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Thermal concomitants and biochemistry of the explosive discharge mechanism of some little known bombardier beetles[1,2]

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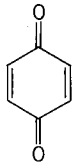
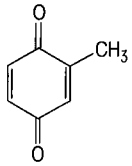
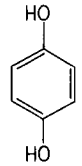
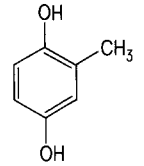
Summary. The quinonoid defensive spray of 2 carabid beetles of the subfamilies Metriinae and Paussinae is ejected hot (55 °C and 65 °C), with a heat content of 0.19 and 0.17 cal/mg. Hydroquinone(s) and hydrogen peroxide are identified as precursors of the quinones, indicating that in these lesser known 'bombardier beetles' the explosive discharge mechanism is similar to that of the familiar bombardiers of the genus *Brachinus* (subfamily Carabinae, tribe Brachinini).

Bombardier beetles include species of 3 different subdivisions of the family Carabidae: a) the tribe Brachinini of the subfamily Carabinae, b) the subfamily Paussinae (including its 2 tribes, the Ozaenini and Paussini), c) the subfamily Metriinae. All discharge 1,4-benzoquinones from their paired abdominal defensive glands, and do so audibly, hence their name[6-9]. Details of the discharge mechanism have been worked out for the Brachinini only, which includes the best known and most commonly collected bombardiers (e.g. the genus *Brachinus*). Hydrogen peroxide and hydroquinones, stored in the sac-like inner chamber of the glands, are passed through a 2nd chamber (the reaction chamber) containing crystalline catalases and peroxidases, causing the hydroquinones to be explosively oxidized to benzoquinones[6,10] and the reacting mixture to be expelled as a hot spray (100 °C)[11]. The question remained whether in the Paussinae and Metriinae the bombarding mechanism might be similar. We here present evidence that it is. Only a single species of the tribe Paussini had previously been studied and shown to generate its quinones in brachinine fashion, from hydrogen peroxide and hydroquinone[12]. No comparable precursor determinations had been made with Ozaenini or Metriinae, nor had thermal measurements been made of the spray of any bombardiers other than Brachinini.

We had available for study several live specimens of

Chemistry of spray, and of the glandular fluid that gives rise to the spray, in the two bombardier beetles studied

	Previously identified principal components of spray[9]			Compounds detected in glandular sac			
			$CH_3(CH_2)_{13}CH_3$			H_2O_2	$CH_3(CH_2)_{13}CH_3$
Subfamily Paussinae (tribe Ozaenini) *Goniotropis nicaraguensis*	+	+	+	+	+	+	+
Subfamily Metriinae *Metrius contractus*	+	−	+	+	−	+	+

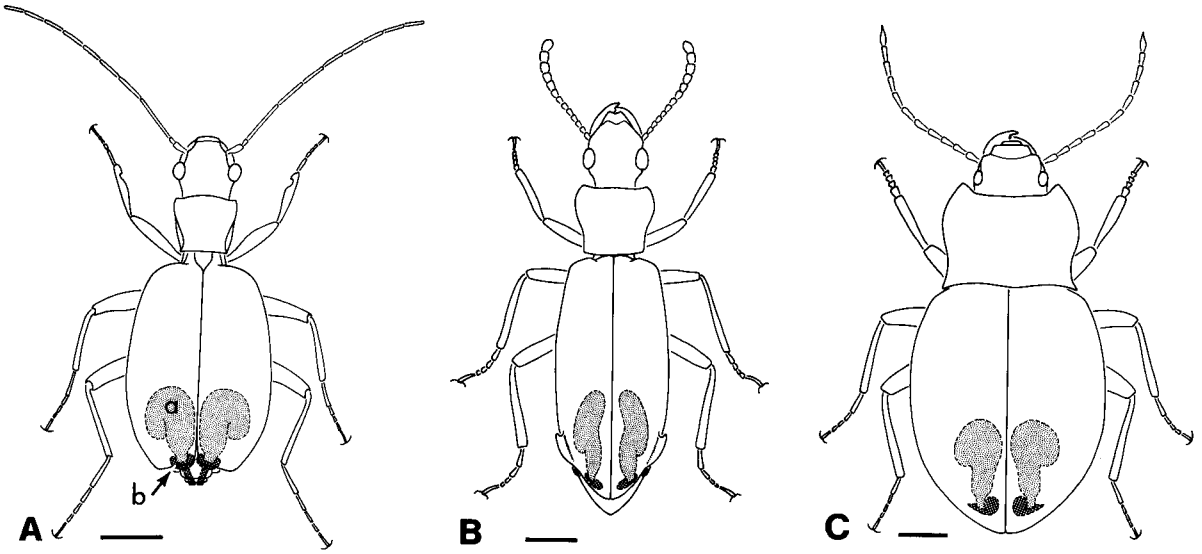


Diagram of the paired defensive glands in the 3 major types of bombardier beetles. *A* Subfamily Carabinae, tribe Brachinini (based on *Brachinus* sp.). *B* Subfamily Paussinae (based on *Platycerozaena panamensis*, but representative also of *Goniotropis nicaraguensis*). *C* Subfamily Metriinae (based on *Metrius contractus*). Storage sacs and reaction chambers, represented respectively by (a) and (b) in A, are comparably shaded in the 3 figures. Omitted from the glands is the tissue, usually lying appressed against the storage sacs, that secretes the sac contents. Reference bars = 2 mm.

Goniotropis nicaraguensis (an ozaenine from Panamá), and of *Metrius contractus* (the rare and only North American metriine, which we obtained from one of its few known collecting sites in California). We had previously shown both species to eject quinones[9]. Measurements of spray temperature (temp.) and heat content (h.c.) were made by causing the animals to discharge upon appropriate electronic sensing devices, as previously described for *Brachinus*[11]. The results (± SD) were similar for the 2 species:

M. contractus: h.c. = 0.19 ± 0.07 cal/mg (N = 5 discharges from 3 beetles) temp. = 55 ± 11 °C (recorded maximum = 77 °C) (N = 27 discharges from 3 beetles)

G. nicaraguensis: h.c. = 0.17 ± 0.06 (N = 3 discharges from 1 beetle) temp. = 65 ± 16 °C (recorded maximum = 87 °C) (N = 10 discharges from 2 beetles)

The thermal properties of the spray were clearly perceptible, as they are in brachinine bombardiers, both when the beetles were handled and caused to discharge on the fingers, and when they were placed momentarily in the mouth.

Dissection showed the glands of *G. nicaraguensis* and *M. contractus* to be fundamentally alike. They are two-chambered, and in that respect comparable to those of the Brachinini (fig.). As was known from other morphological studies[13], non-bombarding carabid beetles, which include the great majority of Carabidae, have single-chambered glands lacking reaction chambers. Such beetles discharge their secretions cold, since the defensive products, which include a broad array of aliphatic and aromatic compounds[7,14] are stored as such in the glands and not synthesized at the moment of ejection.

Prediction had it that in both *G. nicaraguensis* and *M. contractus* the inner chamber of the glands should contain hydrogen peroxide and hydroquinone(s), as well as pentadecane, a hydrocarbon known to be part of the quinonoid spray of both species[9]. This was confirmed. Two beetles of each species were dissected, and their glands excised intact. With each beetle, one gland was transferred to titanium sulfate reagent for hydrogen peroxide analysis, the other to diethyl ether for analysis of hydroquinone(s) and pentadecane.

Hydrogen peroxide was determined spectrophotometrically using the yellow complex formed in the presence of titanium sulfate[15]. Hydroquinones were converted into their bis-trimethylsilyl derivatives (using 'TriSil' reagent), and both they and *n*-pentadecane were quantified by gas chromatographic analysis (5% OV-1 column, 30–200 °C at 8 °C/min) using *n*-tridecane as an internal standard. The percentages of the components were calculated taking the relative response of the flame ionization detector into account.

The results, qualitatively identical for the 2 beetles of each species, are summarized in the table. Meaningful quantitative data were obtained with 1 *Metrius* only, the single specimen that we succeeded in dissecting without causing leakage in the valve separating the 2 compartments of the glands. With the other beetles such leakage could not be prevented, as evidenced by gas evolution, apparent in the glandular sac and indicative of hydrogen peroxide breakdown, which had invariably begun to occur before the glands could be transferred to solvent. With the single *Metrius*, in which sac component levels could be expected to be at their normal high, such levels, expressed as percent of sac content (calculated from gland weight difference before and after rupture in reagent or solvent), were as follows: hydrogen peroxide 24%; hydroquinone 13%; pentadecane 4%. The values for hydrogen peroxide and hydroquinone are in the range of those reported for brachinine bombardiers[6], and clearly in accord with expectation[11], given the thermal properties of the spray.

These data, taken together with those previously reported for the species of *Paussus*[12], demonstrate that the defense mechanisms of Metriinae and Paussinae bear close similarity to that of the Brachinini. Whether these similarities are a consequence of evolutionary convergence or an indication of close phyletic affinity, remains to be seen. In our view, based in part on morphological evidence that we will be presenting elsewhere, bombardier beetles are a monophyletic group in which the ability to bombard evolved only once.

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Sucrose gap longevity is markedly improved by addition of lanthanum to the sucrose

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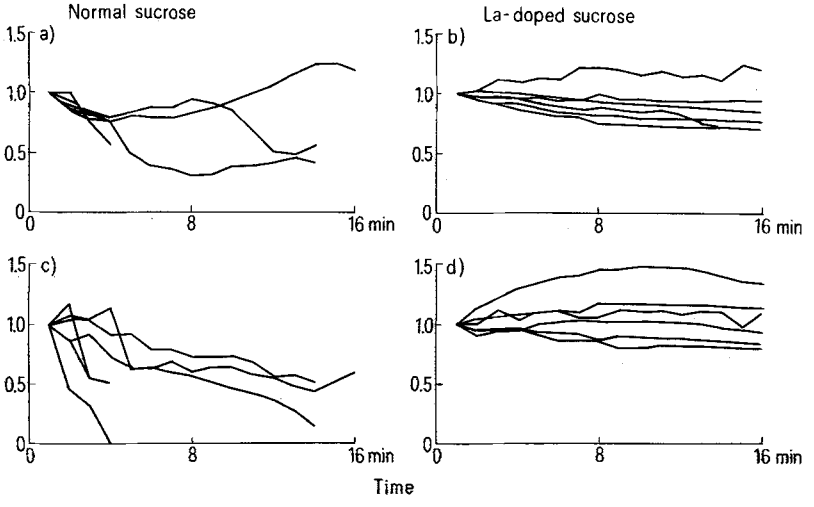
Summary. The addition of low concentrations of lanthanum to the sucrose in the double sucrose gap technique for the study of giant axons significantly prolongs preparation longevity and decreases drift.

The sucrose gap technique for the assessment of membrane potentials on small nerve cells without intracellular electrodes was introduced by Stämpfli[1] and developed for voltage clamping of lobster giant axons by Julian et al.[2,3]. Despite the usefulness of the technique it suffers from the drawback of short preparation viability and high drift. This report describes a dramatic improvement in both longevity and reduction of drift by the simple expedient of doping the sucrose with low concentrations of lanthanum.

In the double sucrose gap technique as applied to giant nerve axons an artificial node is formed by the patch of membrane between the sucrose streams. Many such nodes may be studied on just one axon by translating the whole axon through the experiment chamber in short steps, thereby bringing successive fresh areas of membrane into the region between the sucrose streams. The longevity of any one node is often only a few minutes and rarely exceeds 30 min. During this time the resting potential tends to meander and often falls by tens of mV in abrupt steps. There is usually a corresponding rise in leakage current. Furthermore, the kinetics and magnitude of active sodium and potassium currents studied in voltage clamp usually drift during the life of any one node. If a node deteriorates too much during an experiment one simply translates the axon to form a new node and begins the experiment all over, but it is difficult to do experiments which require comparative measurements spaced more than a few minutes apart.

In 1972 New and Trautwein[4] suggested that low concentrations of calcium in the sucrose improved the stability of muscle preparations in sucrose gap and this was soon found



Normalized peak sodium current for clamp steps to −15 mV (a and b) and resting potential (c and d), assessed at 1-min intervals in normal or La-doped sucrose. Each determination is normalized to the value at 1 min. Three nodes in normal sucrose and 3 nodes in La-doped sucrose from each of 2 axons are shown. In normal sucrose all but 1 experiment had to be terminated before 16 min because the current required to hold the potential at −100 mV went up precipitously. In La-doped sucrose all nodes easily survived 16 min.