The requirement of both EC venom and thrombin for the induction of human lymphocyte fluorescence in the above in vitro system led to the assumption that, in the causation of lymphocyte fluorescence in EC-venom inoculated guinea-pigs[1], thrombin, formed in vivo by the action of the venom procoagulant[4], plays an essential role. Supportive evidence for such a mechanism was provided by the prevention of lymphocyte fluorescence by heparinization of the guinea-pigs prior to injection of EC venom and fluorescein. Furthermore, subsequent intravascular administration of a large amount of thrombin to such heparinized envenomated animals caused the appearance of lymphocyte fluorescence.

The verification of the role of the venom procoagulant in lymphocyte fluorescence induction in an in vitro plasma medium is wrought with the difficulty that the leucocytes are trapped in the clot induced by the venom procoagulant. This obstacle was overcome by using plasma defibrinated by thrombin. In a mixture containing defibrinated plasma instead of serum, EC venom produced lymphocyte fluorescence. Addition of thrombin to this system, in which thrombin is produced by the procoagulant, was not required to obtain cell fluorescence, in contrast to the experiments in which serum was used as medium. Venom procoagulant (0.1 ml, 100 μg protein content/ml) or venom protease fraction (0.1 ml, 1200 μg protein content/ml), each alone, did not induce cell fluorescence in defibrinated plasma medium, but they were able to do so by joint action. Evidently, the fluorescein-staining promoting action of EC venom is due to a combined action of its procoagulant, inducing thrombin formation, and an additional venom component contained in the protease fraction[5].

Résumé. Le venin de l'*Echis colorata* produit la fluorescence des lymphocytes humains dans un mélange contenant du plasma défibrinogéné et de la fluorescéine. L'effet du venin est causé par l'action conjointe du thrombin produit par le procoagulant du venin et d'un autre facteur, qui peut être une protéase.

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The Effect of the Anticoagulant EDTA on Oxygen Uptake by Bone-Marrow Cells

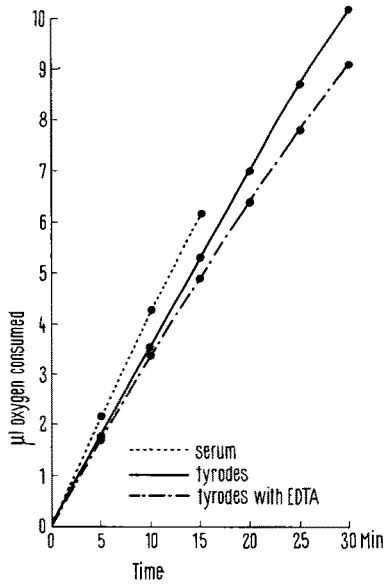
It is generally assumed that low concentrations of anticoagulants such as heparin and ethylenediaminetetraacetate (EDTA) are not excessively damaging to blood cells[1-3]. Studies in this laboratory using a modified Clark oxygen electrode apparatus have demonstrated that the uptake of oxygen by bone-marrow cells is significantly reduced in balanced salt solution containing a low concentration of disodium EDTA. The results of this experimental work are outlined in the present communication.

Methods. Male Holtzman rats, 42 $\pm$ 2 days of age, were used in all experiments. Animals were killed by a sharp blow to the base of the skull and the 2 femora and tibiae removed. The bones were split lengthwise and the marrow teased apart in Tyrode balanced salt solution. The composition of the Tyrode solution was: NaCl 8.0; KCl 0.2; $MgCl_2 \cdot H_2O$ 0.1; $NaH_2PO_4 \cdot H_2O$ 0.5; $NaHCO_3$ 1.0; glucose 1.0 g/l distilled water. In experiments using anticoagulants, the concentration of NaCl in Tyrode solution was reduced by 1.0 g/l and a corresponding amount of disodium EDTA dihydrate powder ($Na_2EDTA \cdot 2H_2O$) was added. This gave a concentration of Na_2EDTA of 1 mg/ml and served to maintain osmolarity of the suspending medium at 310 mOsm/l as measured with a Fiske osmometer.

Cell suspensions were obtained by passing the marrow successively through two 100-mesh stainless steel screens.

Determination of cell counts were made using a Coulter Counter Model B. Aliquots of the marrow cell suspension were diluted to 5 ml with either isologous serum or Tyrode solution with or without Na_2EDTA to obtain the desired number of cells for study.

Oxygen consumption of the bone marrow cells was determined at 37 °C with a Model 53 Biological Oxygen Monitor (Yellow Springs Instrument Company, Ohio).



Comparison of oxygen consumption of rat bone-marrow cells (cell concentration 8×10^7) in different suspending media. Each line represents the mean of 10 experiments.

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Results and discussion. The Figure shows that the oxidative response of bone-marrow cells in Tyrode solution is reduced compared with that of cells in serum. The decrease in oxidative activity of the bone-marrow cells in Tyrode solution was highly significant ($p < 0.01$) after 5 min incubation time. This observation is in good agreement with numerous other studies showing a stimulatory effect of serum on the oxygen uptake by blood cells[4-6]. Addition of Na_2EDTA to Tyrodes solution depressed oxygen consumption of bone-marrow cells. The reduction in oxygen consumption reached the lower significance level ($p < 0.05$) at 15 min incubation and was highly significant ($p < 0.01$) at 20, 25, and 30 min incubation.

The capacity of EDTA to chelate di- and trivalent cations is well established. HILAL et al.[7] reported a reduction in electrostatic charge of lymphocytes and myelocytes of dog bone marrow by low concentrations (0.2%) of EDTA. It seems likely that EDTA would complex with cations at the cell surface and prevent their entrance into the cell interior. This would exert a profound effect on the Na^+/K^+ balance and other cellular events[8]. In view of these considerations it is not surprising to find that EDTA has an inhibitory effect on the oxidative response of bone-marrow cells. Because of the sensitivity of bone-marrow cells to small concentrations of EDTA, the effect of this anticoagulant must be considered in any metabolic studies of these cells.

Résumé. Comparée à celle des cellules en sérum, la réponse oxydative des cellules de moelle osseuse de rat dans une solution de tyrode est réduite. L'addition de disodium éthylénédiaminététracétate dihydrate (Na_2EDTA) à la solution de tyrode abaissa davantage la consommation d'O_2 des cellules de moelle osseuse. Cette réduction atteignit un niveau plus bas ($p < 0{,}05$) à 15 min d'incubation et fut bien marquée ($p < 0{,}01$) à 20, 25 et 30 min d'incubation.

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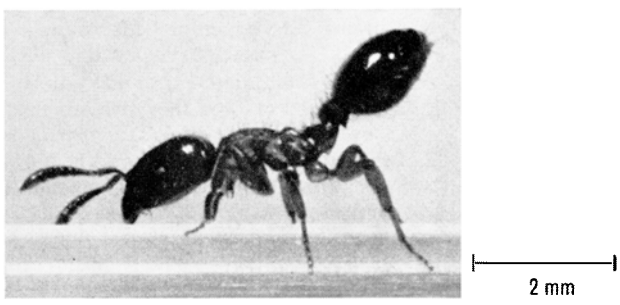
«Locksterzeln» begattungsbereiter ergatoider Weibchen von *Harpagoxenus sublaevis* NYL. (Hymenoptera, Formicidae)

Die geflügelten Geschlechtstiere der meisten Ameisenarten kopulieren auf dem Hochzeitsflug in der Luft. Flügellose, morphologisch der Arbeiterin ähnliche Weibchen kommen besonders bei sozialparasitischen Arten vor. Sie sind die normale Königinnenform der sklavenhaltenden Myrmicine *Harpagoxenus sublaevis*. Neben solchen ergatoiden Weibchen treten bei dieser Art nur selten geflügelte Vollweibchen auf[1,2]. Die Begattung der ergatoiden Weibchen durch die normal geflügelten Männchen kann also nicht in der Luft stattfinden. Nach Laborbeobachtungen erklettern die begattungsbereiten ergatoiden Weibchen in den Abendstunden zwischen 18.00 und 22.00 Uhr erhöhte Punkte in der Nestumgebung und sitzen dort in der abgebildeten Haltung (Figur), wobei die Kloake weit geöffnet und der Stachel vorgestreckt ist, ähnlich wie bei «lock-» oder «giftsterzelnden» Honigbienen[3]. Kommt ein begattungsbereites Männchen etwa 2 bis 3 cm in die Nähe eines solchen Weibchens, läuft es geradewegs darauf zu und reitet auf. Die Kopula dauert insgesamt nur 15–20 sec; das Männchen sinkt während der Verhängung bewegungslos nach hinten. Nach erfolgter Spermaübertragung wendet sich das Weibchen und beisst das Männchen in den Hinterleib, worauf sich die Verhängung löst. Ein Männchen begattete innerhalb einer Stunde 6 ergatoide Weibchen erfolgreich (erwiesen durch Präparation des Receptaculum), die Weibchen kopulierten jeweils nur einmal.



Begattungsbereites ergatoides Weibchen von *Harpagoxenus sublaevis* in der typischen Stellung des «Locksterzelns».

Das geschilderte Lockverhalten wurde bei Ameisen bisher noch nicht beobachtet. Die Anlockung der Männchen erfolgt vermutlich durch einen Duftstoff. Ob dieser aus der bei *Harpagoxenus* im Vergleich zu verwandten Arten ungewöhnlich gross entwickelten Dufourschen Drüse stammt, ist Gegenstand weiterer Untersuchungen.

Summary. Young ergatoid females of the dulotic ant *Harpagoxenus sublaevis* have a special way of attracting males. In swarming time these originally wingless females climb elevated points in the surroundings of their nest. There they sit holding their abdomen upwards, presumably emitting an attracting substance. When a male approaches such a female, copulation can be observed.

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