nition processes (*4*) and cellular adhesion (*5*). Because implantation of CFU-S involves a site-recognition process, we set out to characterize the involvement of some surface peptides and carbohydrates in the CFU-S recognition-implantation process.

Bone marrow suspensions from 6-week-old CF1 mice were prepared in glucose-free Hanks solution (GFH) and passed through a 200-mesh stainless steel screen. Nucleate cells were enumerated by a Coulter counter. Cells at 3.5×10^7 per milliliter were incubated with or without enzyme in GFH for 1 hour at 37°C. All enzymes were assayed for activity (*6*). Trypsin-incubated cells were treated with excess trypsin inhibitor prior to washing. Cells were washed once by a 30-fold dilution in GFH, and resuspended in GFH for injection. Recipient mice received 850 rads (*7*) 24 hours before transfusion of 2.5×10^5 nucleated marrow cells via tail-vein injection. After 7 days, mice were killed, and their spleens were fixed in a mixture of alcohol, formalin, and acetic acid. Colonies were counted under a dissecting microscope at a magnification of ×15.

Table 1 shows the effect of selective removal of cell-surface components on spleen colonies. None of the proteases tested—elastase, Pronase, papain, or trypsin—had any effect on CFU-S. Similarly, α-D- and β-D-glucosidase, β-galactosidase, and α-L-fucosidase had no effect on CFU-S. However, *Vibrio cholerae* neuraminidase (VCN) reduced CFU-S by about one-half (56 percent). Trypan blue viability of the inoculum was unaffected. VCN exhibited no measurable nonspecific enzymatic activities. Heating VCN for 10 minutes in a boiling water bath abolished both enzymatic activity and the reduction of CFU-S, indicating that the action was enzymatic rather than a nonspecific phenomenon. In addition, enzyme concentrations far in excess of that required to achieve maximal reduction in colonies (Table 2) had no additional effect, further supporting the idea that VCN exerts its effect by removal of sialic acid rather than by cytotoxicity.

Surface sialic acid residues, therefore, appear to play a role in the fate of approximately half of the CFU-S in the inoculum. The mechanism of this VCN-induced reduction of spleen colonies is not clear. Several possible explanations, however, could be put forward—that is, killing of some CFU-S, a rapid clearing of VCN-susceptible CFU-S from circulation, or the blocking of some CFU-S from implantation.

Table 2. The effect of *Vibrio cholerae* neuraminidase (VCN) concentration on the number of spleen colonies. Bone marrow cells (3.5×10^7) in 1 ml were incubated with appropriate amounts of VCN for 1 hour at 37°C. After being washed once in GFH, 2.5×10^5 nucleated marrow cells were transfused into lethally irradiated mice. After 7 days mice were killed, their spleens were removed and fixed, and colonies were counted. The results are expressed as the means ± standard error of at least seven spleens.

VCN (units)	Colonies per 2.5×10^5 cells
0	19.4 ± 3.4
0.01	20.0 ± 1.2
0.1	17.4 ± 2.3
0.5	15.2 ± 3.2
1.0	9.7 ± 2.3
25.0	9.9 ± 2.0
50.0	10.2 ± 3.0

Selective killing of some CFU-S could occur if cryptic antigenic sites were exposed by VCN treatment, as occurs with human lymphocytes (*8*). A naturally occurring antibody to these exposed antigens has also been reported (*9*). Similarly, VCN-induced exposure of cryptic sites on a heterogeneous subpopulation of the CFU-S could be a mechanism for selective killing of those CFU-S.

Removal of sialic acid from plasma proteins (*10*) and erythrocytes (*11*) results in their clearance from circulation, and VCN-treated rat lymphocytes exhibit altered circulation patterns (*12*). Therefore, the possibility that VCN-treated CFU-S are removed from circulation or bound at another site must be considered.

Some receptors are exposed on the surface of cells for only a portion of the cell cycle (*13*). If the presence of sialic acid–containing receptor is also a function of cell cycle, VCN could effectively block the implantation of only those CFU-S with exposed implantation receptors. While the actual mechanism of the VCN-induced reduction in spleen colonies is unclear, an important role for surface sialic acid in spleen colony formation is demonstrated.

QUENTIN TONELLI, RUSSEL H. MEINTS
Laboratory of Experimental Hematology, School of Life Sciences, University of Nebraska, Lincoln 68588

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Membrane Potential of Mitochondria Measured with Microelectrodes

Abstract. *The membrane potentials of giant mitochondria from cuprizone-fed mice were found to be independent of metabolic state. Experiments are described in which the presence of the microelectrodes in the inner mitochondrial space, and the metabolic viability of the impaled mitochondria, are validated.*

In some previous studies (*1–4*), membrane potentials were measured in isolated mitochondria (3 to 4 μm in diameter) of *Drosophila*, by means of microelectrodes driven by a piezoelectric drive. The mitochondria were coupled, and the potentials, which ranged from 10 to 20 mv, inside positive, were found not to depend on metabolism (*2*). The membrane resistance ranged from 1 to 4 ohm-

cm². Evidence was presented to support the notion that the microelectrodes were located in the inner mitochondrial space, and that the measured potentials at least approximated those across that of the intact mitochondrial semipermeable membrane. The potentials were found to be sensitive to the osmotic pressure of the medium (*1*), and quantitatively reflected the distribution of carboxylic acids (*3*). Furthermore, the potentials did not decay even after repeated impalements except when the mitochondria were visibly damaged (*4*).

Some objections to these studies have been presented [for example, see (*5, 6*)]. Some of these objections are based on observations that small cells cannot support a true resting potential with a duration exceeding a few milliseconds because of the leakiness induced by the impalement (*5, 7*). The reports of a rapid decay of the resting membrane potentials of Ehrlich ascites cells and *Amphiuma* red blood cells are contradicted by other studies carried out with the same cells (*8*), in which much slower decay rates (several seconds) were found. Furthermore, microelectrode impalement of smaller cells has shown stable membrane potentials for relatively long periods (*9–11*), and in some cases the potentials were shown to respond in a biologically significant manner (*10*). Another objection questions whether the microelectrode is in the inner space of the mitochondria (*6*). The ionic basis of the potential (*3*) which depends on the distribution of carboxylic acids argues against this objection. In addition, in the present study we find a K^+ diffusion potential of a significant magnitude in the presence of valinomycin, in support of the notion that we are in the inner space.

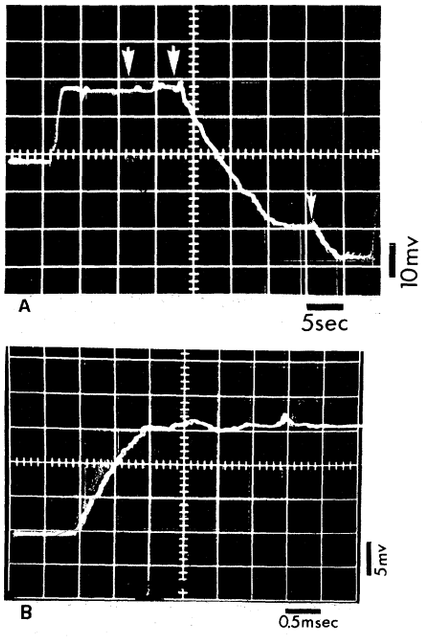

Fig. 1. (A) Oscilloscope trace of impalement of a 5-μm mitochondrion isolated from liver of mouse maintained on a diet supplemented with cuprizone. Mitochondria were suspended in 5 m*M* KCl, 1 m*M* MES, 0.3 osmolal sucrose, *p*H 7.4, at 4°C. The metabolism of endogenous substrates was blocked with rotenone (8×10^{-7} *M*). Tip potential was +3 mv; resistance, 1.8 megohm (1.4 ohm-cm² with the assumption of there being no convolutions of the surface membrane). At the first arrow, succinate was added (to a final concentration of 3 m*M*) by means of a Hamilton syringe. Similarly 1.4×10^{-7} *M* valinomycin and 50 m*M* NaCNS were added at the second and third arrows, respectively. (B) Fast-sweep oscilloscope record of impalement. The mitochondria were suspended in 5 m*M* KCl, 1 m*M* MES, 0.3 osmolal sucrose, *p*H 7.4, to which was added 8×10^{-7} *M* rotenone and 3 m*M* sodium succinate.

In the present study, mice were fed cuprizone as described by Suchy and Cooper (*12*). Mitochondria were isolated from homogenates (0.25*M* sucrose, *p*H 7 to 8) of the liver. Cells, nuclei (swollen), and debris were discarded by centrifugation at 120*g* for 80 seconds. After filtration through a quadruple layer of cheesecloth, the mitochondria were isolated by centrifugation at 3000*g* for 5 minutes. Generally, after isolation the mitochondria were resuspended in 0.3 osmolal sucrose containing 1 m*M* MES [2-(*N*-morpholino)ethanesulfonic acid] and 10 m*M* KCl, *p*H 7.4

A small portion of the mitochondrial population was 5 to 15 μm in diameter. The mitochondria were sandwiched between a thin layer of agar and a glass cover slip (0.15 mm thick). The optical system consisted of a Zeiss Universal microscope equipped for differential interference microscopy (*13*) with a water immersion lens (×40; numerical aperture, 0.75). The mitochondria were impaled manually by means of a Leitz micromanipulator. Measurements of the potential were carried out with a high-input impedance amplifier with a digital voltmeter or an appropriate oscilloscope. The time response of the system was less than 500 μsec. Resistances were measured by passing 10^{-11} amp pulses. The glass micropipettes were pulled on a Chowdhury micropipette puller (*14, 15*). The microelectrode tips were estimated, by means of a scanning electron microscope, to be 0.1 to 0.2 μm and 13° in taper (*16*). The microelectrodes were filled with 2*M* KCl by boiling under vacuum. The resistances of the microelectrodes were between 10 and 80 megohms, and the tip potentials were invariably less than 14 mv. The microelectrodes were connected to an amplifier by a Ag-AgCl bridge. The reference electrode was a Ag-AgCl wire.

Figure 1A shows the oscilloscope trace of the impalement of one mitochondrion in the presence of rotenone. The potential is initially positive (about 17 mv). The first arrow indicates the addition of succinate, which has no significant effect. The second and third arrows indicate the addition of valinomycin and NaSCN, respectively. These additions reverse the polarity of the potential and produce a total decrease of about 45 mv. Figure 1A illustrates (i) the lack of leakage (which would result in the decay of potential) when a rather large change in membrane potential is induced, (ii) the location of the microelectrode in the inner compartment since the potential induced by valinomycin (second arrow) is consistent with a diffusion potential requiring a large internal K^+ concentration, and (iii) the lack of metabolic dependence, since the addition of succinate (first arrow) does not change the potential. We interpret the change in potential after the third arrow as the result of the thiocyanate diffusion potential. The potentials in this experiment (mean of four determinations ± standard deviation) were 17.5 ± 0.5 mv prior to the addition, and 17.0 ± 0.5 mv, −18.5 ± 2.6 mv, and −29.3 ± 2.0 mv after each addition in the order shown in Fig. 1. The changes are ap-

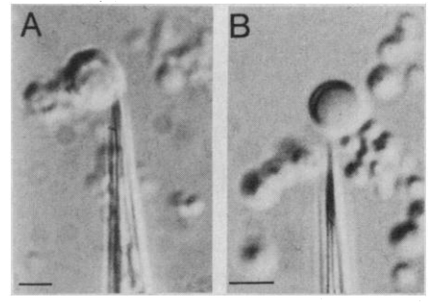

Fig. 2. (A) Giant mitochondrion from cuprizone-fed mice under conditions allowing calcium phosphate accumulation after impalement. Characteristic effects of accumulation were observed only when the mitochondria were in a solution of 2 m*M* $MgCl_2$, 10 m*M* tris-Cl, 2 m*M* NaH_2PO_4, 0.25 osmolal sucrose, 10 m*M* NaCl, 2 m*M* $CaCl_2$, 10 m*M* succinate, 4 m*M* adenosine triphosphate (ATP), *p*H 7.4. In this experiment, ATP was excluded and accumulation was triggered after impalement by adding 4 m*M* ATP. Essentially the same results are obtained by excluding Ca^{2+} from the medium and then adding it as a trigger. The potential did not change over the incubation period of 3 minutes in the presence of ATP. (B) Control. Conditions identical to those in (A) except for the absence of ATP. Identical results were found when the calcium, the phosphate, or the succinate were excluded. The marker corresponds to 5 μm.

proximately the same when the order of the addition is altered or the impalements are carried out after the additions.

Figure 1B shows a fast-sweep oscilloscope trace of an impalement in the presence of succinate. The rate of increase in potential is very rapid (about 1 msec), consistent with the rapid advance of the electrode and the absence of a decay. Also, this rate of increase is consistent with the theoretical expectations from microelectrodes with a sharp taper (*17*). The membrane potentials are independent of the resistance of the electrodes used (and hence tip diameter and electrode taper), suggesting that these measurements are not the result of the leakage of salt from the electrode tip. The mean potentials measured in 30 impalements under identical conditions (1 m*M* KCl, 10 m*M* tris, 0.3 osmolal sucrose, *p*H 7.4, with the potential recorded within 4 seconds) were 16.3 mv. The standard deviation from this value was ± 0.9 mv, although the electrode resistances varied from 10 to about 80 megohm. The constancy of the recorded potentials with time (for example, compare A and B in Fig. 1) also supports the notion that diffusion of electrolyte from the microelectrode tip does not play a significant role in the measured potential.

We have developed an assay to measure the accumulation of calcium phosphate under the conditions of massive loading described by Greenawalt *et al.* (*18*) and, hence, the transducing ability. The accumulation is detected with quantitative interference microscopy. The measurements were made with a ×40 objective and matching condenser (numerical aperture, 0.65) (Jamin-Lebedeff system, Zeiss) according to the deSenarmont method of compensation (*19*). We made measurements by eye to the point of maximum darkness (extinction) for the mitochondria and calculated the optical path difference as described by Forer and Goldman (*20*). The results were consistent with an increase in mitochondrial dry mass of 10.6 ± 0.9 percent (N = 15) for one experiment and 12.9 ± 0.8 percent (N = 22) in another, under conditions facilitating calcium phosphate accumulation. These mitochondria show a granulation with an apparent formation of small granular inclusions as seen with differential interference optics after Nomarski. This response requires both succinate and adenosine triphosphate. In our experiments, this response is blocked by antimycin A or oligomycin.

The mitochondria are still viable after impalement since they can undergo these characteristic changes (see Fig. 2). The potential does not change during the accumulation (*21*).

BRUCE L. MALOFF
STYLIANOS P. SCORDILIS*
HENRY TEDESCHI
Department of Biological Sciences and Neurobiology Center, State University of New York, Albany 12222

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* Present address: Section on Molecular Cardiology, Building 10, Room 7B-15, National Institutes of Health, Bethesda, Md. 20014.

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Weaver Ants: Social Establishment and Maintenance of Territory

Abstract. *Workers of the African weaver ant* Oecophylla longinoda *recruit nestmates to previously unoccupied space by means of odor trails laid from the rectal gland, a hitherto unrecognized musculated organ located at the rear of the rectal sac. When enemy ants and other intruders are encountered on the territory, the* Oecophylla *assemble nestmates into small resting clusters by dispensing an attractant-arrestant pheromone from the sternal gland, a second newly discovered organ located on the last abdominal sternite. Under prolonged stress, additional forces are recruited to the combat area with the aid of the rectal-gland trail substance.*

The weaver ant genus *Oecophylla* consists of two living species, *O. longinoda* of tropical Africa and *O. smaragdina* of southeastern Asia and Melanesia. These insects are renowned for their habit of constructing arboreal nests out of leaves bound together with larval silk. The workers hold small larvae in their mandibles and move them back and forth between the leaf margins like shuttles. Weaver ants are also exceptionally abundant, aggressive, and territorial, a circumstance that makes them of significance in tropical forest ecology and agriculture (*1*). We report here the discovery of a remarkably complex communication system by which *O. longinoda* workers acquire and subsequently control new territorial space. The ants engage in two modes of recruitment, during which they

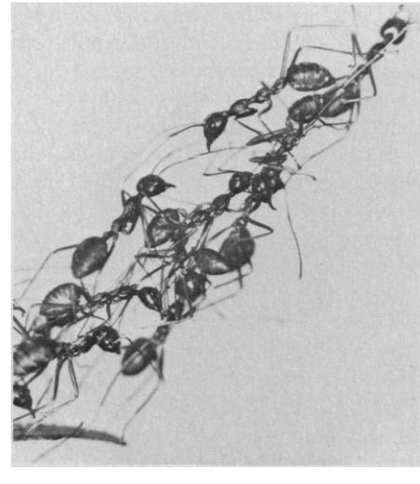

Fig. 1. Workers of the weaver ant *Oecophylla longinoda* form living bridges by linking their bodies into chains. The worker seen running over the upper portion of this bridge is laying an odor trail from her everted rectal gland directly onto the bodies of her nestmates. The trail will guide other members of the colony to a newly available foraging space.