

added exogenously) for the functional presence of the enzyme, the possible need for the continued presence of an unstable prostaglandin was suggested by the data. Taken in conjunction with those studies, the present results lend further support to the hypothesis that the enzyme, fatty acid cyclooxygenase, may in some way be a necessary component for the complete expression of the IFN-mediated anticellular and antiviral states.

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Energetics of Running Cockroaches

Abstract. *Male cockroaches Gromphadorhina portentosa were made to run at 0.03, 0.07, and 0.12 kilometer per hour on a miniature treadmill within a small respirometer. Oxygen consumption was directly related to running velocity. The half-time necessary for oxygen consumption to reach a steady state during exercise was about 1 minute and the half-time for recovery was 4 to 6 minutes. The energetic cost of transport was comparable to that for bipedal and quadrupedal vertebrates.*

The past 10 years have seen heightened interest in comparative locomotion, with an emphasis on the energetics of vertebrates (1-3). Except for flying insects (4), however, little comparable information has been obtained concerning invertebrates. The only quantitative evaluation of the energetics of a terrestrial invertebrate under controlled conditions appears to be that of the land crab *Cardisoma guanhumi* (5). This large species was exercised on a treadmill at specified speeds while wearing a respiratory mask. The experiment revealed several striking parallels between land crab and vertebrate energetics, including a direct relation between oxygen consumption ($\dot{V}O_2$) and running velocity. Moreover, the crab compensated for the increased O_2 demand by increasing ventilation of its gill chamber. The minimum cost of transport (the amount of energy required to move 1 g of animal 1 km) was also similar to that for pedestrian vertebrates (1-3).

We studied the energetics of the large wingless tropical cockroach *Gromphadorhina portentosa* by constructing an airtight Lucite respirometer enclosing an axle-driven latex treadbelt. The belt was driven by a rheostat-controlled d-c gear motor outside the chamber and was capable of a constant speed of approxi-

mately 0.8 to 30 cm/sec. Inflow and outflow gas ports allowed for constant circulation of fresh air through the 125-ml working space of the chamber. Air was continuously drawn through the respirometer at a rate of 56 ml/min. After leaving the chamber, the air passed through a Drierite filter to absorb water before entering one sensor cell and flowmeter of an Applied Electrochemistry S-3A oxygen analyzer coupled to a Linear Instruments model 282 integrating chart recorder. For comparison, room air was drawn at the same rate through a Drierite filter and into a second sensor cell of the analyzer. This permitted us to measure differences in oxygen between room air and gas leaving the respirometer. This value, multiplied by the airflow, gave us the $\dot{V}O_2$ after being corrected to dry air under standard conditions of temperature and pressure (6).

Ten animals with a mean weight of 5.2 ± 0.8 g were run on the treadmill at 0.03, 0.07, and 0.12 km/hour, the latter velocity being near the maximum that can be sustained by this species. Each cockroach was exercised once per day at a single speed in a randomized order over a period of 1 week. We placed the insects in the respirometer for 60 minutes before exercising them, and during the last 15 minutes determined their rest-

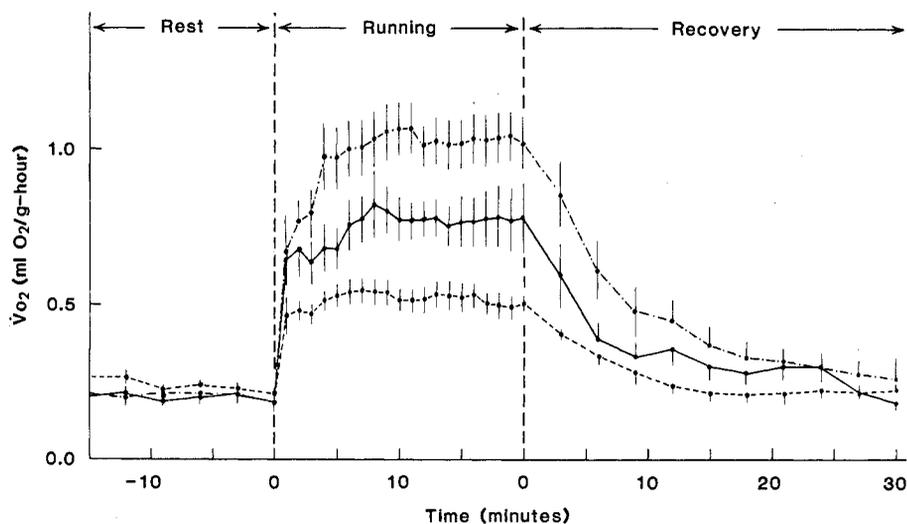


Fig. 1. Mean $\dot{V}O_2$ of cockroaches running on a treadmill for 20 minutes at 0.03 km/hour (bottom record), 0.07 km/hour (middle record), and 0.12 km/hour (top record). The vertical bars represent 95 percent confidence intervals.

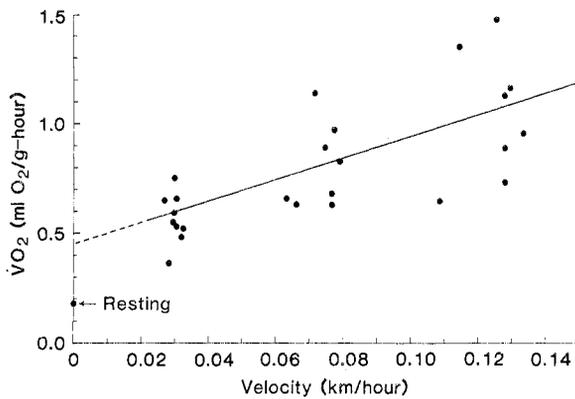


Fig. 2. Steady-state $\dot{V}O_2$ of cockroaches running on a treadmill at different velocities. The y intercept of the regression line is 2.4 times higher than the resting $\dot{V}O_2$.

ing $\dot{V}O_2$. The average value obtained was 0.19 ± 0.05 ml of O_2 per gram per hour, which is comparable to the $\dot{V}O_2$ of other insects (4, 8). Experiments were performed at $24^\circ \pm 1^\circ C$.

After the treadmill was turned on, most individuals readily adjusted, maintaining a position midway in the working section. An experiment was aborted if the roach flipped over, became agitated, or lodged against the rear of the respirometer. Each session lasted 20 minutes, followed by a 60-minute recovery period; $\dot{V}O_2$ was monitored throughout.

All the roaches rapidly responded to the exercise by increasing $\dot{V}O_2$ (Fig. 1). The half-time to reach a steady state was about 1 minute. Steady state itself was achieved within 4 minutes even at the fastest speed. The $\dot{V}O_2$ remained constant during the sessions, with the cockroaches showing no evidence of fatigue in most cases. This rapid rise of $\dot{V}O_2$ to a steady state is similar to that seen in vertebrates and is decidedly different from that seen in crabs (5) and snails (7).

After exercise stopped, $\dot{V}O_2$ remained elevated for different periods depending on the intensity of the exercise. Thus the half-time to recovery was between 4 and 6 minutes, with complete recovery by about 15, 30, and 45 minutes after cessation of the slow, medium, and fast runs, respectively. The length of time that $\dot{V}O_2$ remained above the resting rate is comparable to that observed for birds and mammals but considerably shorter than that for crabs and many lower vertebrates (5, 9, 10). Presumably such variations reflect differences in O_2 delivery mechanism. Birds and mammals have an efficient gas exchange and circulatory system that is adapted for rapid metabolism. Lower vertebrates rely more on anerobic methods to support exercise and thus develop a greater O_2 debt requiring longer recovery periods (9). Crabs, with their rather sluggish circulation and low O_2 carrying capacity, re-

quire a long time to reach steady state $\dot{V}O_2$ during exercise, develop a large O_2 debt, and need long periods to eliminate lactic acid (5). Insects, on the other hand, transport O_2 directly to the cells through the tracheal system. Thus their response to exercise is rapid and the O_2 debt is nominal (4).

In cockroaches $\dot{V}O_2$ during exercise is directly related to the intensity of the exercise (Fig. 2). Interestingly, the highest steady state $\dot{V}O_2$ that we observed was only about five times higher than the resting value. Although somewhat higher $\dot{V}O_2$'s were measured during brief periods of intense activity at speeds up to 0.18 km/hour, all values were significantly lower than those measured for many flying insects, which during activity can maintain $\dot{V}O_2$ rates 25 to 100 times higher than when at rest (4). However, the cockroach metabolic scope is consistent with those of the crab and small vertebrates (5, 9, 11).

Figure 2 shows a least-squares regression line relating $\dot{V}O_2$ to velocity (S). When this line, $\dot{V}O_2 = 0.45 + 4.92S$, is extrapolated back to zero speed, the predicted $\dot{V}O_2$ at rest is 2.4 times higher than the rate actually measured. This discrepancy, noted previously for many terrestrial vertebrates (1, 2), is not understood. It may be due to a "postural cost" of locomotion (2) or to the fact that $\dot{V}O_2$ is not linearly related to velocity (12).

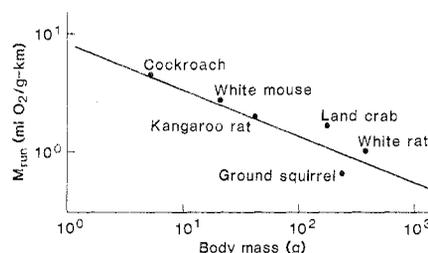


Fig. 3. Minimum cost of transport (M_{run}) for cockroaches, crabs, and several small mammals. The regression line (1) is represented by the equation $M_{run} = 8.46 W^{-0.40}$, where W is body mass in grams.

The slope of the regression line comparing $\dot{V}O_2$ with velocity has been used to compare the minimum cost of transport in different species (1-3). The slope represents the relative rise in $\dot{V}O_2$ with each increase in velocity and permits species with radically different resting metabolic rates to be compared. Figure 3 shows that the data for cockroaches fall close to the regression line for small mammals (4). The same is true for crabs (5). These data also fit the regression line for bipeds and quadrupeds (3). Consequently, it appears that the energetic economy of pedestrian locomotion may be similar among animals of the same size regardless of the number of legs involved or the nature of the circulatory or respiratory system used to supply the aerobic requirements.

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$$Z = 1 - e^{-\dot{V}\Delta t/V} \quad (1)$$

where flow rate (\dot{V}), time (t), and chamber volume (V) are known:

$$Z = \frac{F_t - F_{t-1}}{F_{eq} - F_{t-1}} \quad (2)$$

Combining Eqs. 1 and 2 allows one to solve for the equilibrium value that would eventually be reached if $\dot{V}O_2$ were held constant:

$$F_{eq} = \frac{F_t - F_{t-1}}{1 - e^{-\dot{V}\Delta t/V}} + F_{t-1} \quad (3)$$

Given only two measurements over a brief interval and the "washout" characteristics of the chamber, F_{eq} can be calculated and substituted for F in a standard equation [modified from P. Withers, *J. Appl. Physiol.* **42**, 120 (1977)] to estimate instantaneous $\dot{V}O_2$:

$$\dot{V}O_2 = \dot{V} \frac{(F_1 - F)}{(F_1 - F_{eq})} \quad (4)$$

where F_1 is the fractional influx oxygen concentration.

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Continuous Lines of Basophil/Mast Cells Derived from Normal Mouse Bone Marrow

Abstract. *Nonadherent tissue culture cell lines were established from normal bone marrow of a variety of mouse strains. The lines possessed morphological and histochemical markers of the basophil/mast cell and contained committed stem cells for metachromatic cells. Their derivation from normal marrow and their lack of tumorigenicity despite long-term culture makes these cell lines potentially important for studies of the mechanisms of allergic reactions and inflammation as well as the differentiation pathways involving this subset of hematopoietic cells.*

Techniques for growth of normal hematopoietic cells in culture have recently been developed (1, 2). With the use of these procedures, multipotential stem cells (CFU-s), mixed colony-forming cells, and committed stem cells (CFU-c), including granulocyte/macrophage, erythroid, megakaryocytic, and lymphoid progenitor cells, can be generated in primary culture for up to several months (2-6). However, the establishment of continuous cell lines of normal hematopoietic cells has so far been limited to the lymphocyte series, in which a specific growth factor for T lymphocytes has been isolated and shown to stimulate T cell proliferation in vitro (7, 8). The loss of self-renewal capacity of nonlymphocyte stem cells in culture may be attributable to irreversible, spontaneous differentiation, but the mechanism is unknown.

Certain cells are reported to release growth factors for the proliferation in culture of specific subpopulations of normal hematopoietic cells (9-13). A growth-stimulating factor (or factors), released by the BALB/c mouse myelomonocytic leukemia cell line WEHI-3 (14) has been reported to support continuous growth of mouse bone marrow cells in suspension culture (15). In the present report, we demonstrate the establishment, in WEHI-3-conditioned medium (W-CM) (16), of bone marrow cell lines that have characteristics of basophilic granulocytes or tissue mast cells and contain committed stem cells for metachromatic cells (CFU-META).

Cultures were initiated with bone marrow cells of NIH Swiss and 129/J mice with the use of Greenberger's modification (3) of Dexter's system (2). After 4 to 6 weeks in primary culture, nonadherent

cells in the population were transferred into Dulbecco-Vogt modified minimum essential medium supplemented with 30 percent fetal bovine serum (Colorado Serum Company) and 10 percent W-CM. Transferred cells were maintained at 37°C in 7 percent CO₂ in air. For the first few passages, fibroblast- and macrophage-like cells attached to the surface of the petri dish. However, within five to ten cell transfers at weekly intervals, adherent cells in the culture were eliminated. In the absence of W-CM, nonadherent cells could not be maintained in suspension culture; only fibroblastic adherent cells survived. Optimal concentrations of W-CM for growth of nonadherent cells ranged from 8 to 12 percent.

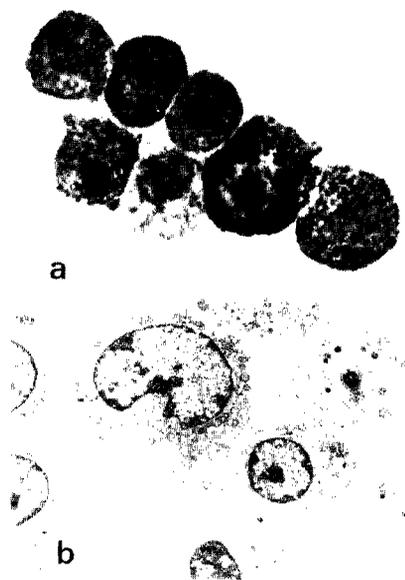


Fig. 1. (a) Photomicrograph of the BM-2 bone marrow cell line stained by Wright-Giemsa ($\times 480$). (b) Electron micrograph of the BM-2 cell line ($\times 5300$).

Cells were routinely passaged weekly at a concentration of 5×10^4 cells per milliliter; the cell doubling time ranged from 50 to 60 hours. Cell lines derived in this manner have been maintained continuously for more than 20 months.

In order to examine the reproducibility of the system, long-term cultures of fresh bone marrow cells from AKR/J, CBA/N, C57BL/6N, C57BL/KsJ, C57L/J, C58/J, 129/J, and NIH Swiss strains of mice were attempted according to the methods described (16). Five to ten individual bone marrow cultures were initiated with each strain. Nonadherent cells were easily passaged and could be maintained indefinitely with NIH Swiss, 129/J, C57BL/6N, and C57BL/KsJ bone marrow cultures. Under the same conditions with the AKR/J, CBA/N, C57L/J, and C58/J strains, it was difficult to maintain nonadherent cells in successive culture for more than 3 months. It has been reported that adipocyte colony formation in primary bone marrow cultures correlates with the ability of such cultures to generate CFU-s and CFU-c of the granulocyte series (3). We observed that adipocyte colony formation in the primary culture appeared to correlate with success in establishing nonadherent bone marrow cell lines.

Typical morphological features of cells in the established cell lines included cytoplasmic granules that stained dark violet to red-purple by the Wright-Giemsa method (Fig. 1a). The nuclei of most cells were round to oval, but segmented nuclei were also observed. Examination by electron microscopy (Fig. 1b) showed that cell surfaces were roughened by microvilli and that the cells invariably contained numerous immature granules. These findings suggested that the established lines consisted of cells of the basophil/myeloid series.

The bone marrow cell lines were next analyzed by histochemical techniques for enzymes characteristic of different hematopoietic subpopulations. The results (Table 1) with representative cell lines indicated the lack of detectable myeloperoxidase, which is normally associated with myeloid cells of the neutrophil series (17). Lysozyme activity, which is generally associated with cells of the monocyte-macrophage series (18), was also absent. In contrast, toluidine blue staining showed that a high percentage of the cells contained metachromatic granules, which are diagnostic for tissue mast cells or basophilic granulocytes (17).

Nonspecific esterase is a histochemical marker reported in monocyte-macrophages (17). Cell lines examined were