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Exercise Enhances Survival Rate in Mice Infected with *Salmonella typhimurium* (41830)

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Abstract. Mice voluntarily trained on exercise wheels for 16–18 days and were then infected with an approximate LD_{50} dose of *Salmonella typhimurium*. These trained mice exhibited a small, but statistically significant (P = 0.037) increase in survival rate (34/77) compared to sedentary control mice (23/79) after 7 days.

The influence of exercise on resistance to infection has been investigated intermittantly since the late 1800s. Acute, forced exercise to exhaustion *after* infecting rats with anthrax bacilli (1) or after infecting rabbits with *Streptococcus pyogenes* (2) resulted in increased mortality compared to infected animals allowed to remain sedentary. However, if several periods of exhaustive exercise *preceded* infection with Type I pneumococcus, reduced mortality was observed in rabbits (3), rats (4), and guinea pigs (5). In all cases, motorized rotating drums were used to walk or run the animals to complete collapse. These early experiments (all prior to 1930) are difficult to interpret since the number of animals used was usually small, endpoints of survival periods were not defined, and ambient conditions (e.g., light cycle, temperature, time of infection) were neither controlled nor in most cases even reported. For example, for small animals such as rats and mice, room temperature (ca. 22°C) represents a cold stress and makes the generation of a fever difficult or even impossible. Since fever appears to be an adaptive response (6, 7), experiments in thermally uncontrolled environments could lead to varying febrile responses and therefore varying resistance to infection. Circadian variations in resistance to infection have also been reported. In rodents, the highest resistance seems to occur at night (8, 9). The failure to control and regulate the above variables could lead to difficulties in interpretation of the data collected.

In the majority of exercise studies following 1930, rodents were forced to swim, often to near drowning. Hirsch (10), and Kreis and Hirsch (11) reported that an intermediate number of swimming sessions after infection resulted in a reduced rate of tubercle bacillus growth in mice compared to sedentary controls. In contrast, mice subjected to either excessive or minimal exposures to the exercise regimen exhibited increased bacillus growth rate over controls. Likewise, Harisch *et al.* (12) found that 5 days of swimming stress imposed before infection with *Pasteurella multocida* increased survival in a small group of rats while fewer or more numerous swimming periods appeared to be detrimental to survival. Friman *et al.* (13), however, found forced swimming to have no effect on numbers of lesions, bacteria counts, or antibody responses in rats after infection with *Francisella tularensis.*

Forced exercise *after* viral infection apparently reduces resistance in mice. Exhaustion in rotating drums increased paralysis and mortality to poliomyelitis (14). Forced swimming increased coxsackie B-3 virus replication, increased mouse mortality (15), and reduced interferon production (16).

In addition to the studies on infectious microorganisms listed above, the effect of exercise on tumors in mice has also been described. Rusch and Kline (17) observed retarded fibrosarcoma growth after forced exercise training in rotating drums. Rashkis (18) demonstrated that swimming training before inoculation with ascites tumors increased survival rate and that this rate remained higher than that of control mice as long as the daily swimming sessions continued.

Tentative hypotheses might be drawn from the above work that: (i) moderate exercise *prior* to infection may be beneficial in terms of resistance to bacterial infection, and (ii) exercise *after* contracting an infection, especially exhaustive exercise, may be detrimental. We have tested the first hypothesis with a large

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number of mice under controlled ambient conditions using a voluntary exercise protocol which is not likely to inflict severe psychological stresses which can affect immune function (19, 20). We report that mice maintained in their thermoneutral zone and allowed free access to exercise wheels for 16–18 days exhibited a significantly higher survival rate than untrained age-matched control mice when infected with *Salmonella typhimurium*.

Materials and Methods. Male CD-1 mice (specific pathogen free) were purchased from Charles River Laboratories in Portage, Michigan and were randomly assigned to individual cages. Twenty mice were allowed to train in cages containing 15-cm diameter activity wheels. Five wheels were fitted with microswitches connected to electronic counters to monitor wheel revolutions. The trained mice reached a steady activity rate of 4.2 ± 1.0 (SEM) km per night by the 10th night of the training period. An equal number of mice were placed in cages without wheels to serve as controls. All animals were maintained on a 12-hr light/dark photoperiod in an environmental chamber between 28 and 30°C (within the thermoneutral zone for mice) (21) at 20% relative humidity. The mice had free access to rodent chow (Purina 5001) and water. To encourage appropriate activity patterns further, food for the untrained mice was placed on the cage floor while food for the trained animals was placed in stainless-steel feeders, requiring the mice to climb approximately 10 cm.

After 16–18 days, both groups were given intraperitoneal injections of approx 5×10^6 *S. typhimurium* (LT-2 wild type, American Type Culture) in 0.1 ml phosphate-buffered saline. In preliminary studies, injection of this concentration of bacteria at midnight into group-caged sedentary mice produced about a 50% mortality rate after 7 days. Bacteria stocks were stored in glycerol at −20°C, then grown on blood–agar plates at 37°C for 16 hr prior to infection. Bacteria concentrations were estimated using McFarland barium sulfate turbidity standards (Difco Laboratories) and viable cell numbers were verified by plating dilutions of the bacteria suspensions on blood–agar. The injections were administered between 11 PM and midnight, during the active period of the mice, hence the most likely period of infection under natural conditions. Survival was monitored for 7 days following infection and differences between survival in the two groups were evaluated by Fisher's exact probability test (22).

Results. Immediately prior to infection, the body weights of the trained mice were approximately 5% lower than those of the sedentary controls ($P < 0.001$, Table I). The rectal temperatures of these mice were significantly higher ($P < 0.001$) than the temperatures of the controls when measured during the active period of the animals (Table I).

The mice became lethargic after inoculation with the bacteria. Activity dropped off to 1.4 ± 1.1 km the night following infection; many of the mice stopped running completely.

In three of four repetitions of the experiment, the trained group of mice exhibited a greater survival rate than untrained controls (Table I). Cumulative data for all four experiments are shown in Fig. 1; a small but sig-

TABLE I. BODY WEIGHT AND RECTAL TEMPERATURE OF CD-1 MICE WERE MEASURED IMMEDIATELY BEFORE INFECTION (BETWEEN 11 PM AND MIDNIGHT) AND DIFFERENCES BETWEEN GROUPS DETERMINED BY STUDENT'S *t* TEST[a]

Expt	Body weight (g)		Rectal temperature (°C)		*Log dose of bacteria*	Survival rate	
	Untrained	Trained	Untrained	Trained		Untrained	Trained
1	29.0 ± 0.3	28.3 ± 0.4	38.4 ± 0.1	39.4 ± 0.1	6.77	5/19	10/19
2	31.2 ± 0.6	30.5 ± 0.3	38.4 ± 0.1	39.3 ± 0.1	6.78	7/20	11/19
3	31.6 ± 0.4	29.2 ± 0.4	38.4 ± 0.1	39.1 ± 0.1	6.83	3/20	6/19
4	31.3 ± 0.5	28.7 ± 0.4	38.3 ± 0.1	39.4 ± 0.1	6.34	8/20	7/20
Cumulative data	30.8 ± 0.3	29.2 ± 0.2	38.4 ± 0.1	39.3 ± 0.1		23/79	34/77
	$P < 0.001$		$P < 0.001$			$P = 0.037$	

[a] Survival rates were determined 7 days after infection and differences determined by Fisher's exact probability test.

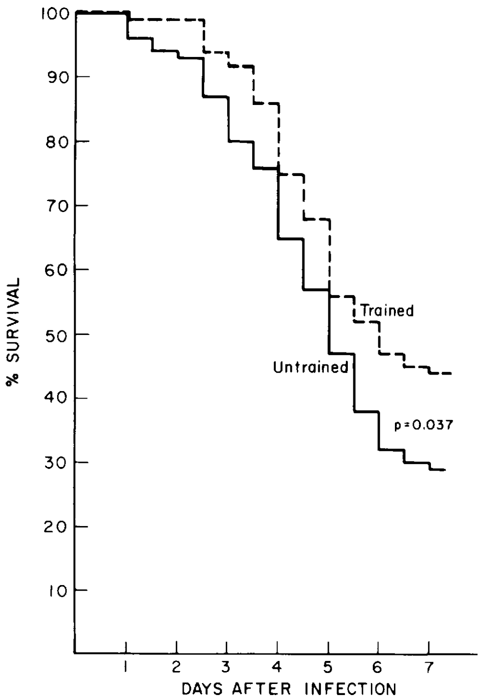

FIG. 1. Survival of male CD-1 mice infected with *Salmonella typhimurium* between 11 PM and midnight (N = 79 untrained mice, 77 trained mice). Statistical significance determined by Fisher's exact probability test.

nificant difference ($P = 0.037$) in survival was observed at 7 days.

Discussion. Clearly, the absence of an exercise wheel does not eliminate spontaneous activity by mice. However, the higher midnight rectal temperature and lower body weight of the trained mice indicates significantly higher spontaneous activity by these mice than by the untrained mice (Table I).

The mechanism responsible for the increased survival to *S. typhimurium* in these mice after voluntary exercise training is unknown. Studies involving human subjects indicate that exercise increases blood neutrophil concentration (23), K cell activity (24, 25), and acute phase protein synthesis (26). A recent report (27) suggests that exercise may cause the release of endogenous pyrogen, the mediator of many nonspecific host defense mechanisms.

Nonimmunological adaptations may contribute to enhance survival as well. For example, exercise-induced alterations in energy metabolism may be beneficial to the host or detrimental to the pathogen (28). Another possibility may be that the cardiovascular conditioning accompanying training provides protection against endotoxic shock associated with bacteremia (29).

The critical influence of the environment was exemplified in one experiment (not included in Table I or Fig. 1) when ambient temperature fell to 22°C after infection due to equipment failure. The survival rate of the trained mice in this cold stress (4 of 20 survived) was less than half that of control mice (9 of 20 survived) under the same environmental conditions.

Before one could generalize that voluntary exercise training provides enhanced resistance to infection, these experiments would need to be repeated using other pathogens. It is obviously impossible to extrapolate these results to exercise in human beings, not only because of possible species differences, but because these data were collected under carefully controlled laboratory conditions with the mice able to select freely their exercise intensity. Human beings tend to exercise in uncontrolled, often inclement conditions, and the exercise intensity and duration is often influenced by external factors relating to intraspecific competition.

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