

Effect of Antiorthostatic Suspension on Interferon- $\alpha/\beta$  Production by the Mouse (41939)

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*Abstract.* Mice were suspended in a model that simulates weightlessness that occurs during prolonged space flight. After 1 and 2 weeks of suspension in an antiorthostatic (head-down tilt) position, the mice were challenged with polyriboinosinic-polyribocytidylic acid to induce interferon- $\alpha/\beta$ . Interferon production was severely reduced in mice that had been suspended. When mice were allowed to recover in cages for a week following removal from suspension, they recovered their full interferon-production capacity. Mice suspended in an orthostatic (horizontal) position did not have their interferon production capabilities affected, which indicates that stress per se was not a major component in the effects of antiorthostatic suspension on interferon induction. © 1984 Society for Experimental Biology and Medicine.

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Weightlessness has been shown to markedly affect cell-mediated immune responses in animals and humans following prolonged space flight (1-4). These effects include lymphoid organ involution and altered rates of blastogenesis. To study these and other generalized effects of weightlessness on biological systems, several animal models utilizing suspension have been developed to simulate the effects of and responses to weightlessness (5-8). These models have been shown to induce fluid electrolyte and musculoskeletal responses similar to those observed during prolonged weightlessness. The fluid/electrolyte alterations have been attributed (5-8) to fluid shifts resulting from antiorthostatic (head-down tilt) positioning, while hindlimb hypokinesia (reduced limb movement) and hypodynamia (reduced mechanical loading) are suggested to underlie the marked musculoskeletal responses.

There have been only two limited applications of rat suspension models to studies of immune responses. One reason the rat model has not been used extensively for studies of the immune system is the relatively limited availability of reagents for study of the immune responses in general and the interferon system, in particular, in rats. Caren

*et al.* (9), utilizing an early version of the suspension model, reported little or no effect of suspension on serum immunoglobulin levels, anti-sheep red blood cell antibody titers, differential leukocyte counts, or spleen weight. These authors did note a pronounced reduction in thymus weight, which could have been the result of the stress associated with the suspension technique rather than a response to suspension itself. Sonnenfeld *et al.* (10) have reported preliminary studies suggesting that suspension of rats results in a suppression of the capacity for interferon production. This study, however, was limited in scope and the experimental design did not permit a complete or accurate interpretation of the results.

We recently characterized a whole body suspension technique for mice that induced physiological effects comparable to those observed in suspended rats and in animals exposed to weightlessness (11). In the present study we extend the previous limited observations and report the results of the first utilization of the mouse suspension model to determine the effects of simulated weightlessness on production of interferon- $\alpha/\beta$ , an important component in the immunological defense of the body (12). The results demonstrate that suspension inhibits interferon production and that stress alone cannot account for the entire response. In addition, recovery following suspension is associated

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with an enhanced capacity for interferon production.

**Materials and Methods.** *Mice.* Female Cox (Swiss) mice were obtained from Laboratory Supply Company, Indianapolis, Indiana. Mice were maintained in an AAALAC-accredited facility under the supervision of a veterinarian.

*Suspension model.* Mice were suspended in an antiorthostatic position (head-down tilt of 15°) for 1 or 2 weeks (11). The hindlimbs were positioned to eliminate load bearing but to permit freedom of movement. This suspension technique has been shown to produce physiological changes similar to those observed during weightlessness (11). Some animals were removed from suspension after 1 or 2 weeks and allowed to recover in metabolism cages for 1 week. Control mice were housed in metabolism cages. Food and water were available to all mice *ad libitum*.

*Stress production.* In order to determine the role of stress in the observed effects, a protocol was initiated that produced stress but did not involve antiorthostatic suspension. In this case, mice were suspended in harnesses for 1 week without the 15° head-down tilt required for antiorthostasis.

*Interferon induction.* Interferon- $\alpha/\beta$  was induced once in each animal by iv injection of 10  $\mu\text{g}$  of poly(I-C) into mice following 1 or 2 weeks of suspension or following 7 days of recovery from 1 or 2 weeks of suspension. Interferon production was induced in control mice following either 1 or 2 weeks in metabolism cages. Since interferon titers did not statistically differ between 1- and 2-week metabolism cage control groups, the data were pooled for statistical comparisons with suspension and recovery groups. The poly(I-C) was formed by annealing polyriboinosinic and polyribocytidylic acids (P-L Biochemicals, Milwaukee, Wisc.) by heating at 45°C for 1 hr (13). The animals were bled 6 hr after injection of poly(I-C), the peak time of interferon production. Interferon produced in this fashion has been previously characterized as interferon- $\alpha/\beta$  due to pH 2 stability, 56°C lability, and sensitivity to an antibody directed against interferon- $\alpha/\beta$  (14).

*Interferon assay.* The antiviral titer of the test sample believed to contain interferon activity was determined by means of a plaque

reduction assay on mouse L-929 cells using the Indiana strain of vesicular stomatitis virus as the test virus (15). The interferon titer corresponded to the reciprocal of the greatest dilution of test sample that reduced virus plaques by 50%. One interferon antiviral unit in this assay was equivalent to 0.88 NIH G-002-904-511 reference unit.

*Statistical analysis.* All experiments contained at least four mice in each group. Since the interferon assay is a titration and a biological assay, a twofold difference in titer had to be observed (50% decrease or 100% increase) before any effect could reliably be determined. Statistical significance was determined by a two-tailed Student *t* test with a  $\alpha$  set at  $P \leq 0.05$  in an a priori fashion.

**Results.** Mice were suspended for a period of 1 or 2 weeks with a 15° head-down tilt. Interferon was induced with poly(I-C); the mice were resuspended for 6 hr and then bled. Sera were assayed for interferon activity. Mice suspended in this antiorthostatic fashion exhibited a significant 70 to 76% decrease in interferon production as compared to controls maintained in metabolism cages (Table I). When mice were allowed to recover in metabolism cages for 1 week after suspension, the mice regained full interferon-production capacity (Table II). In fact, a rebound effect appears to have occurred with enhanced interferon- $\alpha/\beta$  production capacity after recovery (Table II).

To determine if the stress of antiorthostatic suspension could have contributed to the observed inhibition of interferon induction, mice were suspended for 1 week without the 15° head-down tilt (orthostatic suspension). Orthostatic suspension had no significant effect on the production of interferon- $\alpha/\beta$  (Table III).

TABLE I. EFFECT OF ANTIORTHOSTATIC SUSPENSION ON INTERFERON- $\alpha/\beta$  INDUCTION

Treatment	Interferon titer <sup>a</sup>	N	%Change	P
Metabolism cages	654 ± 145	10	—	—
1-week suspension	159 ± 54	4	-76	<0.05
2-week suspension	195 ± 110	7	-70	<0.05

<sup>a</sup> Antiviral titer ± standard deviation.

**Discussion.** Weightlessness has been associated with pronounced effects on the immune system. Several investigations have focused on responses of the immune system in rats to simulation of the effects of weightlessness. Few studies have been undertaken with animal models, however, to investigate the time course and reversibility of these effects as well as the role of stress and positioning. The results of the present study, which utilized a suspension system for simulation of the effects of weightlessness (11), indicate that suspension in an antiorthostatic position can result in inhibition of the capacity for interferon production in mice. This response is absent in animals suspended in an orthostatic position and is not mimicked by the stress of cold exposure. In addition the suppression of interferon inducibility is reversed following removal from suspension.

Suspension of mice for 1 or 2 weeks results in significant inhibition of interferon production when challenged with poly(I-C). This effect was noted much earlier in this study than in the previous study with rats, which suggests that the effects of suspension on the immune system were both rapid and sustained. The effects of simulated weightlessness appear to be transient, as mice allowed to recover for 1 week in metabolism cages following suspension recovered their capacity for production of interferon. In fact, the capacity for interferon induction was significantly enhanced in animals allowed a 1-week recovery period. Thus, the suspension effects can rapidly be reversed.

The fluid/electrolyte responses of suspended rats are dependent upon the orientation of the animal to the gravitational

TABLE III. LACK OF EFFECT OF CONTROL TREATMENT ON INTERFERON- $\alpha/\beta$  INDUCTION

Treatment	Interferon titer <sup>a</sup>	N	%Change	P
Metabolism cages	654 $\pm$ 145	10	—	—
Orthostatic suspension	1101 $\pm$ 224	7	+68	NS

<sup>a</sup> Antiviral titer  $\pm$  standard deviation.

vector, i.e., only animals suspended in an antiorthostatic (head-down) position respond with diuresis and natriuresis (5). In contrast, animals suspended in an orthostatic (horizontal) position are not significantly different from controls with regard to water and sodium excretion. We examined whether an analogous differentiation could be made on the basis of inducibility of interferon. Mice suspended in an orthostatic position were not significantly different from metabolism cage controls in their capacity for interferon production. This is in sharp contrast to the marked inhibition noted in animals suspended in an antiorthostatic position. The mechanisms underlying the effects of antiorthostatic suspension are unknown, but it is now clear that they are due to position and probably are not due to the stress of suspension alone.

In summary, these results suggest that prolonged weightlessness may lead to a transient inhibition of the capacity for interferon production and to a possible increase in the susceptibility to infectious diseases in astronauts or experimental animal subjects. The mouse suspension model appears to be a useful technique to study this effect and the response of other immunological parameters to weightlessness.

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TABLE II. EFFECT OF RECOVERY FROM ANTIORTHOSTATIC SUSPENSION ON INTERFERON- $\alpha/\beta$  INDUCTION

Treatment	Interferon titer <sup>a</sup>	N	%Change	P
Metabolism cages	654 $\pm$ 145	10	—	—
1-week suspension, 1-week recovery	1922 $\pm$ 272	8	+193	<0.05
2-weeks suspension, 1-week recovery	2272 $\pm$ 229	6	+247	<0.05

<sup>a</sup> Antiviral titer  $\pm$  standard deviation.

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