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5. Day 1 of the estrous cycle was characterized by the voluminous postovulatory vaginal discharge. Each of five intact hamsters housed in LD 14 : 10 showed the scalloping pattern. Visual inspection of the chart records was made without prior reference to the estrous cycle data. The mean number of daily revolutions during three consecutive cycles did not vary significantly with stage of the cycle ($.05 < P < .10$, analysis of variance).
6. Scalloping has been repeatedly observed in experiments conducted over a period of more than 2 years; however, it is by no means manifested by all normally cycling hamsters.
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8. Steroid hormones were administered in Silastic capsules (Dow-Corning) [0.062 inch, inside diameter; 0.125 inch, outside diameter (1 inch = .254 mm)] sealed with Silastic adhesive (Dow-Corning type A). Crystalline EB was packed in capsules of about 10 mm functional length. The corresponding length of the progesterone capsule was 20 mm. All surgical procedures were performed under sodium pentobarbital anesthesia (80 mg per kilogram of body weight, injected intraperitoneally).
9. Illumination intensity during the light phase was about 130 lux at the cage level.
10. Differences in the amount of wheel running were not significant among the groups during the pretreatment period; each treatment induced an approximately 20 percent decrease in the number of wheel revolutions. In each of seven hamsters tested, removal of the EB capsules delayed the phase of wheel running by delaying its onset. Similar delays occurred in only 3 of the 12 animals upon removal of the empty or progesterone capsules ($P < .005$, Fisher exact probability test).
11. The interval (mean ± standard error of the mean) between blinding and the insertion of the capsules was 61.7 ± 15.8 days for the empty-capsule group and 52.4 ± 6.3 days for the group given EB capsules.
12. The activity records were pasted day below preceding day. The circadian period was measured by fitting a straight line by eye through the onset of activity. Two of us (L.P.M. and K.M.F.) independently calculated τ during the 20 days that preceded each manipulation. The τ for the treatment interval was calculated for a block of 20 consecutive days after the period of the activity rhythm had stabilized. In six instances, shorter intervals (between 9 and 18 days) were used to estimate τ. Estimates were made without reference to prior or subsequent activity patterns. The two estimators were in agreement to within .04 hour on 39 of the 42 estimates; the remaining three estimates were reanalyzed independently. The mean of the two independent estimates was used in all analyses.
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14. For two hamsters the aftereffect lasted 78 and 82 days, respectively, at which time the animals died. In a third animal the aftereffect lasted for 105 days after capsule removal, at which time the experiment was terminated.
15. In blind-ovariectomized hamsters, EB significantly increased the daily number of wheel revolutions ($P < .05$, t-test). Blank implants did not produce significant changes, and progesterone implants significantly decreased activity.
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Stress-Induced Modulation of the Immune Response

Abstract. *After mice were exposed to a daily auditory stressor for varying lengths of time, the responses of their splenic lymphoid cells in vitro were assessed. Both the blastogenic activity of concanavalin A or lipopolysaccharide and the ability of immune lymphocytes to lyse P815 target cells showed the same patterns of immunosuppression and enhancement.*

While the immunosuppressive properties of short-term exposures to various stressors have been well established (*1*), the effects of long-term stress on the immune response are less clear (*2*). To further elucidate the latter interaction, we have assessed in vitro the responses of lymphoid cells obtained from mice subjected to various periods of environmental stress. The immunoresponsiveness of splenic lymphocytes was evaluated by determining their blastogenic activity following mitogen stimulation (*3*), and by the ability of splenic lymphocytes obtained from mice immunized in vivo to kill P815 target cells (*4*).

Male mice (7 to 12 weeks old) of the AKR or $C57/Bl_6$ strains were used. The mice (four per cage) were subjected to a broad band noise at about 100 db daily for 5 seconds every minute during a 1- or 3-hour period around midnight, at the height of the diurnal activity cycle. Unstimulated controls were exposed only to the normal activity of the animal room. The controls and experimental mice were killed at the same time. Their spleens were removed, and suspensions of splenocytes were prepared. Erythrocytes were lysed with 0.83 percent NH_4Cl buffered to *p*H 7.5 with tris for 10 minutes and then washed in Hanks balanced salt solution. Cells were resuspended and maintained in RPMI 1640 and 10 percent heat-inactivated fetal calf serum.

Both assays of immunologic reactivity, one nonspecific and the other specific, showed the same temporal pattern of hypo- and hyperresponsiveness (Fig. 1), the data having been confirmed in further replications. The activities of both B- and T-cells (derived from bone marrow and thymus, respectively) appeared to be affected similarly: B-cell function as reflected by stimulation with lipopolysaccharide (LPS), and T-cell function as reflected both by stimulation with concanavalin A (Con A) and by the specific lysis of P815 target cells.

The clearest demonstration of the stress-induced modulation of immune function is found in Fib. 1B. Short-term exposure of the animals to the sound stressor (initiated during the week preceding or following immunization with a T-dependent antigen) clearly depressed the lymphocyte-mediated cytotoxic response, while enhancement occurred with longer exposures to sound stress.

Assays of plasma cortisol (*5*) in similarly stressed $C57/Bl_6$ mice (Fig. 2A) show that there is an increase in the circulating levels of this adrenal corticosteroid corresponding to the depression of the immunologic function, and not apparently associated with the enhancement. Daily exposure to the sound stress for more than 10 days produces an adaptation which brings cortisol to base-line levels. However, acute stress increases the blood cortisol to concentrations that are able to suppress the immunologic response to antigens introduced during this period. Coincident with the heightened steroid level is a decrease in the number of viable nucleated cells recovered per spleen from the mice subjected to short-term stress compared to the number recovered from the nonstressed controls (Fig. 2B). This result corresponds to similar decreases in lymphocyte cytotoxicity and spleen index after the administration of exogenous hydrocortisone, as reported by Fernandes *et al.* (*6*). Such depletion of lymphoid cells could occur as a result of either cortisone-induced toxicity or changes in lymphocyte migration patterns (*7*). It is interesting that there is a comparable de-

crease of cells during the period of immunopotentiation when cortisol levels are normal.

The ability of environmental stimuli to enhance as well as to depress immunoreactivity has not been fully appreciated. Certainly, the available evidence supports the view that the stress-induced immunosuppression is mediated through the action of cortisone upon lymphocytes. What of the potentiation of the immune response? That it has not been well documented may be a reflection of differences in assay systems and sampling intervals, as well as in the qualitative and quantitative parameters of stress. For example, stimulation of suckling mice significantly shortens the time they survive after receiving a transplantable lymphoid leukemia (*8*), while the same type of neonatal stress in handled rats significantly increases the amount of antibody produced in response to an injection of nonreplicating antigen (*9*). On the other hand, rats stressed by overcrowding for 1 week prior to immunization by that same nonreplicating flagellar antigen have significantly lower antibody levels than uncrowded animals (*10*), while splenic lymphocytes from rats crowded for 5 weeks prior to immunization with human thyroglobulin showed, in vitro, an increased incorporation of [^{3}H]thymidine when they were stimulated with that antigen (*11*).

Gisler and Schenkel-Hulliger (*12*) have shown that an intact pituitary is necessary for the recovery from adrenocorticotropic hormone (ACTH)-stimulated depression of plaque-forming cells in vitro following immunization with sheep erythrocytes in vivo. A hypophysectomized mouse would not regain normal immune responsiveness unless the administration of ACTH was preceded by receipt of somatotropic hormone (STH). This hormone is but one of the many humoral factors whose concentrations are increased by long-term exposure to environmental stressors (*13*). Therefore, we propose that the enhancement phenomenon reported herein may be due to the elevation of one or more such circulating factors which stimulate lymphocyte reactivity through activation of guanosine 3′,5′-monophosphate, a cyclic nucleotide that is involved in lymphocyte proliferation, and which can be induced by any of a number of neurohumors (*14*).

In summary, we have shown that environmental stressors not only can depress immune responsiveness, but can also enhance it. Both suppression and potentiation appear to be associated with a decrease in the number of viable nucleated splenocytes. Whether this loss is due to the cells' interacting with humoral factors, or to humoral factors causing a change in migration patterns, remains to be elucidated. Levels of cortisol in plasma appear to be temporally related to immunological hyporeactivity but not to hyperreactivity. The mechanism for this latter phenomenon is now open for inquiry.

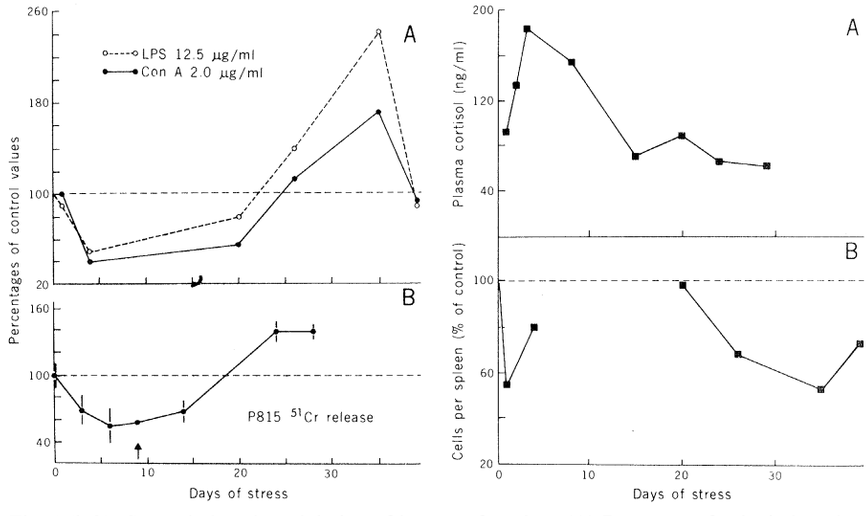

Fig. 1 (left). Stress-induced modulation of immune function. (A) Response of splenic lymphocytes in vitro to mitogens LPS or Con A following stimulation (in vivo) of mice with sound stress for 1 hour per day for up to 39 days. Each point represents the mean of triplicate replications from a pool of two spleens per datum. For control animals, the mean number of counts per minute were 9078 for LPS and 59,368 for Con A. The [^{3}H]thymidine uptake for cells not treated with mitogens ranged from 128 to 400 count/min. (B) Ability of immune lymphocytes to kill target cells in vitro following stimulation (in vivo) of mice with sound stress for 3 hours per day for up to 28 days. Each point represents the mean of triplicate replications from each of two individual spleens. Vertical bars show the range of values. Only one spleen was used at day 9. Arrow indicates that mice were immunized with P815 cells 9 days before they were killed. Some animals had their daily sound stress periods initiated prior to immunization (represented by points to the right of the arrow) while others had their stress sessions started after immunization (represented by points to the left of the arrow). All animals were killed at the same time and the lytic activities of their splenic lymphocytes were assessed simultaneously. The mean lysis in control experiments was 23 percent. Fig. 2 (right). Humoral and splenic changes induced by long-term exposure to sound stress. (A) Plasma cortisol levels of mice subjected to sound stress for 3 hours per day for up to 28 days. (B) Viable nucleated splenocytes recovered per spleen from mice subjected to sound stress for 1 hour per day for up to 39 days. Each point represents the average of two mice.

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4. For the lymphocyte cytotoxicity assay, spleen cell suspensions from C57/Bl$_6$ mice were prepared as previously described. However, 9 days before they were killed, the animals received 3×10^7 DBA/2 mastocytoma (P815) cells, intraperitoneally. Immune or nonimmune splenocytes were incubated with 10×10^3 ^{51}Cr-labeled P815 target cells at a ratio of 100 : 1 for 16 hours at 37°C in a 5 percent CO_2 atmosphere. The amount of ^{51}Cr released into the media was determined in a Packard gammaspectrometer and the percentage of specific lysis was calculated from [(counts released by immune cells minus counts released by the nonimmune cells) divided by (total input counts of ^{51}Cr)] × 100.
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