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Response of Adrenals, Thymus, Spleen and Leucocytes to Shuttle Box and Confinement Stress.* (25772)

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Previously reported studies indicated that exposure of mice to stress-induced emotional disturbance of 2-4 weeks of daily sessions in shuttle box or of physical restraint increase their susceptibility to herpes simplex and Coxsackie B1 viruses(1,2). In contrast, susceptibility to anaphylaxis(3) and to homograft reaction, delayed hypersensitivity(4), decreases after one or more exposures to shuttle box stress. The nature of changes in host-response has not been determined. However, known sensitivity of the pituitary-adrenal, and thymico-lymphatic and blood leucocytic systems to all forms of stress(5), together with the importance of these systems in resistance to infection(6), suggests that they may be related to changes in resistance following stress exposure. Our report describes responses of the above systems in mice exposed to stressors mentioned, as reflected by changes in adrenal, thymus, and spleen weights, and blood leucocytes. These measures were chosen primarily to elucidate mechanisms related to resistance but with the hope that results might also provide other criteria by which stress response could be measured.

Materials and methods. The stressors used, shuttle box and confinement, have been described. The latter stressor, in contrast to shuttle box, tends to minimize activity and avoids shock. Four to 6-week-old female Swiss mice were used(1). Experimental animals were subjected to stress by one of the methods indicated for 6 hours/day, 6 days/week. Control animals were maintained in home cages from which food and water were withdrawn for 6 hours daily. Subgroups of 10 animals and 10 controls were sacrificed after varying intervals of stress exposure, and varying periods of recovery following termination of 28 days exposure to stress. Animals were sacrificed under anesthesia usually within 4 hours after the last stress period. Controls were sacrificed in late morning or early afternoon of days on which stressed animals were killed. Organ weights were determined and expressed as mg/g of mouse weight determined immediately before death. For histological examination, organs were fixed in Bouin's solution, imbedded in paraffin, sectioned and stained with hematoxylin and eosin. Total leucocyte counts were made on tail blood. Differential leucocyte counts were made after May-Gruenwald Giemsa staining. Two hundred leucocytes were counted and separated into the following categories: Polymorphonuclear leucocytes, normal lymphocytes, stress lymphocytes(7), monocytes and eosinophiles.

Results. Shuttle box stress. The data summarized for shuttle box in Table I and in Fig. 1-4 represent composite means from several experiments with the following numbers of animals at intervals indicated: (1) during stress, 20 at 1 d.; 20 at 30 d.; 40 at 21 d. and 110 at 28 d., and (2) during recovery following 28 d. stress, 20 at 7, 14 and 21 d. Adrenal response to shuttle box stress is rapid, hypertrophy becoming evident within 3 days (Fig. 1). Thereafter, the curve for stressed animals rises more slowly through remaining 25 days

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TABLE I. Comparison of Effects of 2 Stressors on Organ Weights and Total WBC of Animals Stressed for Various Periods. 10 animals/series.

Measure of stress / Response and type of stressor	Period of stress: 7 days Median	7 days Range	14 days Median	14 days Range	28 days Median	28 days Range
Adrenal wt/body wt						
Shuttle box stress	.344	.24-.43†	.328	.17-.43*	.405	.34-.53*
Control	.243	.16-.33	.268	.17-.40	.338	.21-.59
Confinement stress	.379	.24-.52†	.316	.18-.49	.324	.24-.44
Control	.250	.17-.30	.256	.14-.42	.242	.18-.46
Spleen wt/body wt						
Shuttle box stress	3.34	1.9-6.0	2.33	1.9-3.9†	2.63	1.5-4.6†
Control	4.14	3.2-5.1	4.14	3.3-5.2	4.84	4.0-7.3
Confinement stress	2.16	1.7-3.2	2.56	1.7-3.8	2.68	2.2-4.4
Control	2.90	1.8-3.9	2.82	2.0-4.0	3.22	1.7-5.8
Thymus wt/body wt						
Shuttle box stress	2.53	.8-3.7*	1.98	1.3-3.4†	2.49	1.4-3.5*
Control	3.17	2.5-3.7	2.99	2.8-3.8	3.08	2.4-3.6
Confinement stress	1.97	1.5-3.1†	1.82	1.5-3.1	2.08	1.0-2.9*
Control	2.96	2.1-4.4	2.52	1.5-3.5	2.96	2.0-6.7
Total WBC						
Shuttle box stress	3,650	1,500- 7,500†	3,600	3,200- 4,800†	3,050	2,000-10,600†
Control	7,100	5,100-14,200	7,100	4,600-10,500	11,400	6,500-22,700
Confinement stress	3,000	1,150- 7,300†	3,275	1,900- 5,300†	3,150	3,000-12,800*
Control	9,100	6,500-13,900	8,200	5,000- 9,700	8,800	2,750-21,900

* p value of .05 to .02 by Mann-Whitney U test. † P value of .01 or greater by Mann-Whitney U test.

of stress. Differences between stressed and control adrenal values increase in significance from 7 through 28 days of stress exposure. Following stress termination, differences slowly decrease to non-significant levels. Microscopic examination indicated that stress-induced enlargement of adrenals was the result of cortical hypertrophy.

The leucocyte drop occurs even more rapidly than does adrenal hypertrophy, in some experiments after a single period of stress (Fig. 2). Difference between groups is well established at 3 days, and leucocyte values for stressed mice continue to fall throughout the 28 day stress. Recovery of leucocyte values towards control levels is rapid following stress termination. While mean differences persist, they are not significant during recovery period. The fall in total count was accounted for by decreases of polymorphonuclear leucocytes and lymphocytes, with the latter showing most pronounced changes. Increases in stress lymphocytes were observed in both stressed and control animals and were not consistently related to experimental stressors.

Spleen weights (Fig. 3) undergo initial rise following a single 6 hour period of stress. Following subsequent stress exposure progressive involution occurs which is gradual, becoming significant at 14 days and maximally significant at 28 days of stress. During recovery both stress and control values decrease significantly but differences between stress and control values are negligible. Histologically, the decrease in size of spleen was associated with marked atrophy of germinal centers of the corpuscles.

Fig. 4 shows changes in thymus weights similar to those in spleen. The initial rise following a single period of stress is again observed. Gradual involution follows continuing stress, with significant differences between experimental and control values apparent at 14 to 28 days. A tendency not statistically significant for continued decreases in both control and experimental weights occurred during recovery. Histological changes in the thymus were less pronounced than in adrenal and spleen; a tendency toward atrophy of the cortex was seen.

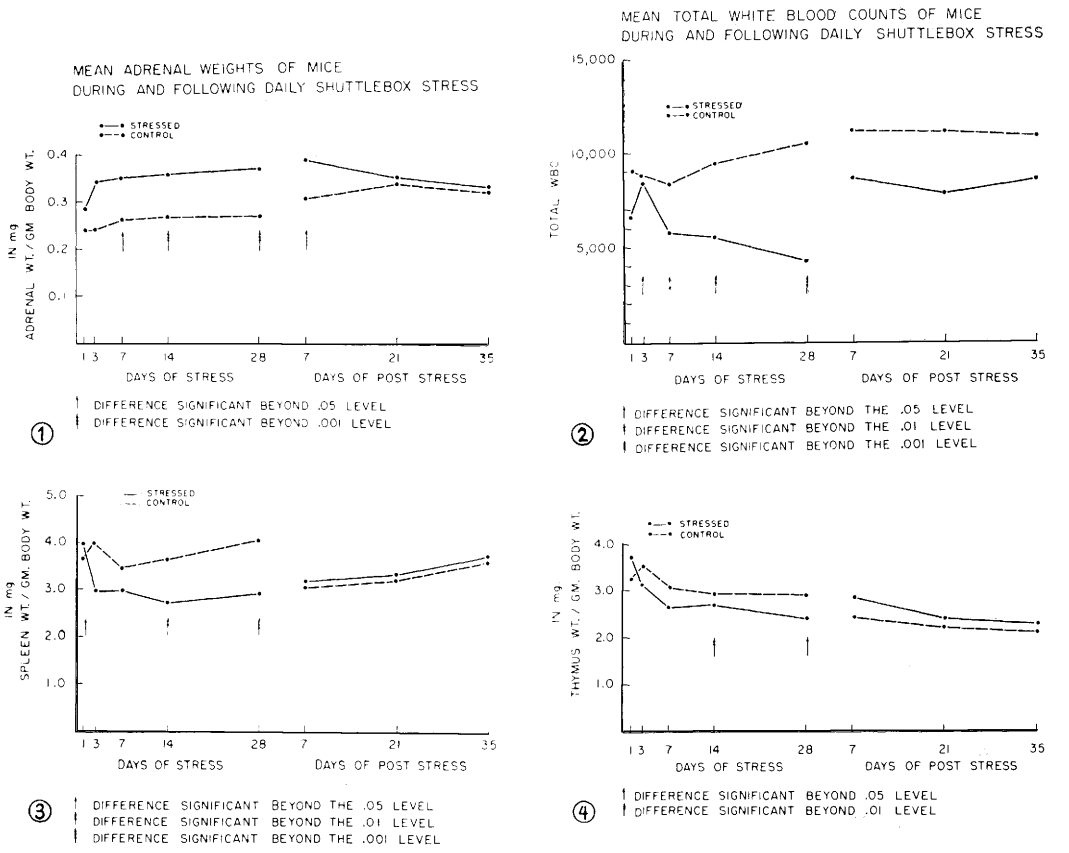

FIG. 1. Mean adrenal weights of mice during and following daily shuttlebox stress.
FIG. 2. Mean total white blood counts of mice during and following daily shuttlebox stress.
FIG. 3. Mean spleen weights of mice during and following daily shuttlebox stress.
FIG. 4. Mean thymus weights of mice during and following daily shuttlebox stress.

Confinement stress. Organ weights and leucocyte changes were also observed in mice subjected to daily stress by confinement for 7, 14 and 28 days and data compared with shuttle box stress studies (Table I). Leucocyte and organ changes are similar in direction but smaller in magnitude and shorter in duration than changes following shuttle box stress.

Discussion. These experiments show pronounced changes in adrenal, thymus, spleen and blood leucocytes following stress of intermittent exposure either to restraint or to shuttle box training. Confinement apparently elicits changes as rapidly as shuttle box exposure, more rapidly in thymus involution; but mice tend to adapt more rapidly and more completely to restraint. This observation is supported by results of previous studies employing experimental infection(1).

Leucocyte count is most sensitive of stress response measures utilized. Changes occur consistently and more rapidly than do other measures following initial stress exposure (Fig. 2); magnitude of change is greater; and values return more rapidly following termination of stress.

That 2 dissimilar stressors elicit similar organ and leucocyte changes suggests a common mechanism. Inasmuch as confinement does not involve electrical shock, and curtails rather than enforces activity, it appears that neither electrical shock nor physical activity is crucial but that fear produced in both situations is the important factor in changes observed. Fear-motivated avoidance response is well known to resist extinction(8), and may account for the more intensive and prolonged effects elicited by shuttle box stress.

Some variation in control values is evident, (Fig. 2, 3, 4). These changes parallel, but to a lesser degree, those observed in stressed mice and may be attributed to extraneous stresses such as noises, temperature variation, individual caging and handling as has been shown by Day, *et al.*(9). The decrease in spleen and particularly thymus weights in controls during recovery is probably related to maturation.

Results differ in some respects from organ and blood changes frequently observed following stress(5). Most investigators report neutrophilic leucocytosis, and relative lymphopenia and eosinopenia. In contrast, our data show leucopenia (Fig. 2), following initial shuttle box stress, which persists through 28 days of stress and which may be related to differences in stressor application. Most studies utilized a single, short exposure to severe stressors or continuous exposure to milder stressors while our mild stressors are intermittent. Selye(5) has noted that leucopenia may follow severe stress before leucocytosis ensues and that leucopenia persists until death in animals that do not survive the alarm reaction. Our stressors, however, are neither severe nor fatal.

Perhaps relevant is the observation of Harlow and Selye(10) that counts taken immediately after exposing rats to cold show leucopenia, while leucocytosis follows in 48 hours. Our counts are ordinarily taken immediately following exposure to stress but consistent leucocytosis is not seen up to 35 days following last exposure.

Also not generally reported is initial rise in thymus and spleen weights, possibly a result of edema noted early in stress responses in these organs(11). Partial recovery of spleen and thymus weights seen during the resistance phase by others is not prominent.

Of changes observed, the rapidly occurring, persistent hypertrophy of adrenals most closely approximates stress response observed by others(5). This response appears not to account directly for increased susceptibility to viral infection, seen after 2 weeks and most marked after 4 weeks of stress(1,2). Rather, splenic atrophy is more nearly related, temporally, to susceptibility to viral infection, suggesting that impaired reticulo-endothelial activity may be an important factor. Immediate adrenal response may explain the resistance to anaphylaxis in acutely and chronically stressed animals(3).

Summary. Changes in organ weights and leucocytes following daily exposure to emotionally disturbing shuttle box or confinement stress were consistently observed. Adrenal hypertrophy and drops in circulating leucocytes were relatively rapid with significant changes observed following 3 to 7 days of stress. Involution of thymus and spleen occurred more slowly, with differences becoming maximum following 14 to 28 days of stress. Differences between experimental and control values returned to non-significant levels in 21 days following termination of stress.

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