

Changes in leukocyte levels associated with social-rearing condition in C57BL/10J mice*

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Thirty-two male C57BL/10J mice were either isolated or pair-reared from weaning until 62-71 days of age. Half of those in each rearing condition were then placed in an arena with a similarly reared mouse, following which they were cut and the total circulating leukocytes counted. The other half of the Ss were cut without being run in the arena. The results showed that leukocyte levels decreased reliably ($p < .001$) by groups: isolate, pair-reared, isolate/arena-tested, pair-reared/arena-tested. No fighting occurred in the arena. It is suggested that isolation-induced aggression is less likely to occur where isolation does not serve as a stressor.

Isolation-induced fighting in male mice has been a widely observed phenomenon, to the point where it seems to have become recognized as a species-common characteristic (e.g., Garattini et al, 1969; Welch & Welch, 1969; several earlier studies are reviewed by Scott, 1966). According to Conner: "Following a period of social isolation, male mice will engage in a characteristic behavioral sequence with one another which usually culminates in an attack [1972, p. 211]."

One mechanism proposed for this behavior pattern by Conner (1972) and others is that there is reduced activity in the pituitary-adrenal system, especially adrenocorticotrophic hormone (ACTH), in isolated mice. Exposure to a greatly changed stimulus situation, such as exposure to other mice, would result in a greater degree of stress than in group-reared mice, resulting in a higher degree of ACTH output.

The study reported here, the first in a series dealing with social rearing conditions, agonistic behavior, stress, and the genetic factors associated with these variables, was designed to determine the stress levels present in isolated vs pair-reared mice, and the differences in these levels when these differentially reared animals are placed in an arena with a similarly reared mouse.

Changes in total circulating leukocyte level was used as our major stress index, since it has been demonstrated that a reliable decrease in the number of leukocytes (leukopenia) occurs for a period of time following application of a stressor (Jensen, 1969). Leukopenia, in turn, has been shown to be directly related to increases in adreno-cortical activity (Gordon, 1955). Changes in leukocyte level are not so rapid as to be affected by the procedure used for obtaining blood.

METHOD

Ss were 32 male mice of the highly inbred C57BL/10J strain,

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between 62 and 71 days of age when tested. When weaned at 21 days of age, half of the Ss (pair-reared group) were placed in pairs into standard plastic mouse cages, the other half (isolation group) were placed in cages alone. Temperature in the colony and testing rooms was maintained at 21°-23°C, and food and water was available to Ss ad lib at all times until they were tested.

For half of the Ss (8 pair-reared, 8 isolates), total white blood cell (WBC) counts were made immediately after 43-50 days under their specified rearing conditions. Each of the remaining Ss (8 pair-reared, 8 isolates) were placed in a 20-cm square arena together with a similarly-reared noncagemate companion for 15 min, followed 25 min later by a WBC count.

Blood sampling procedures were identical for all Ss, and all samples were taken between 9 a.m. and 12 noon.

In order to dilate the blood vessels, each S was exposed to heat from two 40-W bulbs placed directly over the top of the home cage for 5 min. The S was then placed in a small Plexiglas mouse holder, the tail grasped firmly at the tip, and a small cut was made dorsally across the base of the tail with a scalpel blade, and the first two drops of blood were wiped off. The third drop was sucked into a diluting pipette when it beaded, and was mixed for several seconds with Gower's solution. Each sample then filled both sides of an AO Spencer Bright-Line hemacytometer, and leukocytes were counted under a microscope with a 10X objective and a 10X eyepiece. Two Es counted each sample, each counting total leukocytes on one-half of the hemacytometer. Samples on which counts on the two halves of the hemacytometer differed by more than 10% were discarded.

RESULTS AND DISCUSSION

Total leukocytes differ considerably between groups. Ranked in order of increasing leukocyte levels, mice in the pair-raised/arena-tested condition showed the lowest leukocyte level, followed by isolate-reared/arena-tested Ss, pair-reared animals, and isolate-reared Ss. These differences were highly significant when tested by a Kruskal-Wallis analysis of variance by ranks ($H = 32.94$, $df = 3$, $p < .001$). There was little overlap between leukocyte levels between groups. It should be noted that no fighting occurred in the arena, by either pair-raised or isolated animals.

The total absence of fighting in either the isolated or pair-reared mice would throw doubt on the view that

Table 1
Total Leukocyte Counts by Condition

Rearing/Test Condition	N	Mean	SD	Range
Isolated/None	8	10,622*	2,066	9,020-11,600 (7 of 8 Ss Between 10,040 and 11,600)
Pair Reared/None	8	10,020*	2,274	9,320-12,040 (7 of 8 Ss Between 9,320 and 10,080)
Isolated/Arena	8	8,503†	1,612	7,500-9,080
Pair Reared/Arena	8	6,865†	3,896	4,740-9,380 (7 of 8 Ss Between 4,740 and 7,920)

*Mann-Whitney $U = 7, p = .005$

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isolation-induced aggression is a species-common behavior, although it is commonly found in several strains. The consistent finding here of a relative diminution of leukopenia in the isolate animals, compared with those group-reared, both with and without arena testing, provides no lack of support for the role of stress-related hormones in agonistic behavior,

but does point out that the environmental conditions and treatments which might influence them are genotype-dependent.

Further studies are currently in progress, or planned, investigating the same variables as those reported here in a more aggressive strain of mouse, in their F_1 hybrids (with C57BL/10), as well as with other strains and phenomena related to social rearing and stress.

REFERENCES

- Conner, R. L. Hormones, biogenic amines, and aggression. In S. Levine (Ed.), *Hormones and behavior*. New York: Academic Press, 1972. Pp. 209-233.
- Garattini, S., Giacalone, E., & Valzelli, L. Biochemical changes during isolation-induced aggressiveness in mice. In S. Garattini and E. B. Sigg (Eds.), *Aggressive behaviour*. New York: Wiley, 1969. Pp. 179-187.
- Gordon, A. S. Some aspects of hormonal influences upon the leukocytes. *Annals of the New York Academy of Sciences*, 1955, 59, 907-927.
- Jensen, M. M. Changes in leukocyte counts associated with various stressors. *Journal of Reticuloendothelial Society*, 1969, 6, 457-465.
- Scott, J. P. Agonistic behavior of mice and rats: A review. *American Zoologist*, 1966, 6, 683-701.
- Welch, B. L., & Welch, A. S. Aggression and the biogenic amine neurohumors. In S. Garattini and E. B. Sigg (Eds.), *Aggressive behaviour*. New York: Wiley, 1969. Pp. 188-202.

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