Splenic Extramedullary Hematopoiesis in Grouped Male Mice.* (28576)

JOHN P. RAPP AND JOHN J. CHRISTIAN†
Penrose Research Laboratory, Zoological Society of Philadelphia and Dept. Pathology, University of Pennsylvania, Philadelphia

Splenic enlargement due to excessive erythropoiesis has been observed as a result of crowding in voles(1). The purposes of the present article are to: 1) emphasize the participation of megakaryocytic and granulocytic elements as well as RBC forming elements in splenic enlargement in crowded male mice, 2) demonstrate a relationship between splenic extramedullary hematopoiesis (EMH) and splenic weight, 3) show that splenic weight and splenic EMH increase only in the subordinate animals of a group, 4) show that severity of skin fight wounds is related to splenic EMH and splenic weight.

Materials and methods. Male, brown, NMRI strain mice, originally derived from laboratory-bred and reared wild mice were individually isolated at weaning. At 16 weeks of age they were randomly placed into 8 groups of 3 mice per cage; 24 controls were kept individually isolated. Since severe fighting ensued with the grouped mice, they were grouped only from 9:00 a.m. to 5:00 p.m. Monday through Friday and were isolated individually at all other times. Controls were isolated individually at all times. The dominant animal in each group was selected by observing the mice while fighting, the dominant mouse usually "winning" in combat.

All mice were weighed and killed at 19 weeks of age (3 weeks after grouping) and fixed in formalin. Fixed weights for spleen, thymus, adrenal, testes, seminal vesicles, and preputial glands were obtained.

Splenic histology was studied with hematoxylin and eosin stain. Sections were randomized and graded without knowing the identity of the section on an arbitrary scale from 0 to 5 for extramedullary hematopoieses (EMH). Zero was no or minimal EMH, 1 was occasional isolated foci of hematopoietic cells around connective tissue trabeculae, 2 was 1-3 layers of hematopoietic cells around connective tissue trabeculae, 3 was more than 3 layers of hematopoietic cells around trabeculae but not extensive spreading between trabeculae, 4 was like 3 but with additional sheets of hematopoietic cells between trabeculae, 5 was a histological picture like bone marrow. Half grades (*e.g.*, 5, 1.5, etc.) could also be distinguished.

Skin fight wounds were graded on an arbitrary scale: zero was no wounds, 1 a few wounds, 2 several wounds, and 3 many wounds.

Results. Organ weight changes in grouped animals were those usually found for this type of grouping experiment(2). That is, the grouped animals showed significant decreases (by the "t" test) in weights of reproductive organs (testis, seminal vesicles) and thymus as compared to isolated animals. Adrenal weight increased in grouped animals, but the increase was not significant in this experiment.

There were marked differences between grouped and isolated animals for splenic EMH and splenic weight; data are tabulated in Table I. In analyzing the EMH grades a non-parametric test, the Wilcoxon 2-sample rank test(3), was used. For splenic weight the "t" test was used. With both tests the grouped *vs* isolated comparison was made between the 8 cage means for grouped animals and the 24 isolated mice; the dominant *vs* subordinant comparison between the 8 dominant and the 8 cage means for subordinant animals; the dominant *vs* isolated comparison between the 8 dominant and the 24 isolated animals; and the subordinant *vs* isolated between the 8 cage means for subordinant animals and the 24 isolated animals.

From Table I it is obvious that the increased splenic weight in grouped mice was caused by the response of the subordinant

* Supported in part by USPHS grant and by the Naval Med. Research Inst., Bethesda, Md.

† Present addess: Div. of Endocrinology & Reproduction, Res. Labs., Albert Einstein Med. Ctr., Philadelphia.

TABLE I. Splenic Weight and Splenic Extramedullary Hematopoiesis (EMH) for Grouped and Isolated Male Mice.

	Splenic EMH (arbitrary grade)	Splenic wt (mg)
Grouped	2.9 ± .5*	164.2 ± 33.5*
Dominant	1.4 ± 1.0	88.8 ± 22.3
Subordinant	3.7 ± .8	212.7 ± 51.1
Isolated	1.9 ± 1.4	89.0 ± 24.0
Grouped *vs* isolated	.084†	<.005†
Dominant *vs* subordinant	.002	<.005
" " isolated	.560	>.5
Subordinant *vs* isolated	.008	<.005

* Mean ± standard deviation.
† Level of significance for 2-sided test. EMH was tested using the Wilcoxon 2-sample rank test and splenic weight by the "t" test.

animals, the dominant mice not differing significantly from isolated ones (this can also be seen graphically in Fig. 1).

Fig. 1 presents the relationship between splenic EMH and log splenic weight. The Spearman rank correlation coefficient‡(4) for EMH *vs* Spleen weight§ was +.79 which was significant at the $p < .0005$ level (one-sided test).

The data also suggested a relationship between the splenic weight range at each EMH grade and the EMH grade as shown below:

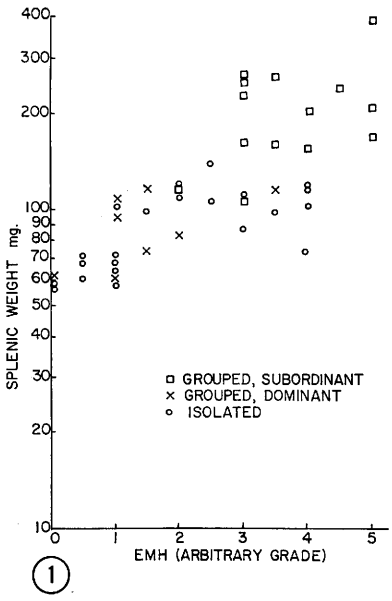
EMH	Splenic wt range (mg)
0	13.5
1	56.6
2	58.7
3	175.7
4	163.2
5	221.5

The Spearman rank correlation coefficient for EMH grade *vs* splenic weight range was +.97 which was significant at the $p < .01$ level (one sided test).

There was no relationship between splenic weight and thymus, adrenal, seminal vesicle, or preputial gland weight in subordinant grouped animals (*i.e.*, those that showed a change in splenic weight). But there was a negative linear relationship between splenic weight and testis weight in subordinant grouped animals. This relationship also was present for testis weight/body weight *vs*

‡ A non-parametric statistic for correlation between 2 variables.

§ Ranking spleen weight or log spleen weight in increasing order gives rise to the same order and thus the same rank correlation coefficient.



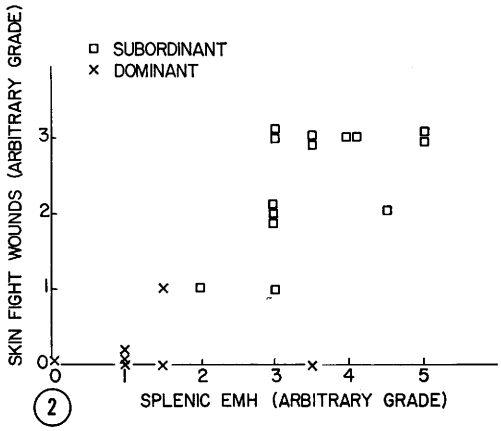


FIG. 1. Splenic weight on a log scale is plotted against splenic extramedullary hematopoiesis (EMH) graded on an arbitrary scale from 0 to 5 (see text for description of grades). The Spearman rank correlation coefficient = +.79 and is significant at $p < .0005$ (one-sided test).

FIG. 2. Severity of skin fight wounds graded on an arbitrary scale from 0 to 3 is plotted against splenic extramedullary hematopoiesis (EMH) graded on an arbitrary scale from 0 to 5. See text for description of grades used in both scales. The Spearman rank correlation coefficient = +.78, which is significant at $p < .01$ (one-sided test).

splenic weight/body weight.‖

Fig. 2 shows a relationship between splenic EMH and severity of skin fight wounds. The Spearman rank correlation coefficient for splenic EMH and skin fight wounds was +.78 which was significant at $p < .01$ (one sided test). There was also an approximately linear relationship between skin fight wounds and splenic weight, as one might expect from comparing Figs. 1 and 2, but fight wounds *vs* testis, thymus, adrenal, seminal vesicle, or preputial gland weights showed no obvious relationship.

Discussion. The splenic hematopoiesis occurring in subordinant grouped mice was very obvious and is constantly seen in this type of experiment. This splenic hematopoiesis involved hyperplasia of erythrocytic, granulocytic, and megakarocytic elements. It should, of course, be noted that a small degree of splenic hematopoiesis is normal in adult mice (5; see also the splenic EMH grade for isolated mice in Table I).

The cause of increased splenic EMH with grouping is unknown, but two obvious possibilities can be suggested. First, there is evidence for increased adrenal function in grouped mice(2) and the pituitary-adrenal axis is known to influence hematopoietic activity in rodents(6,7,8). Therefore changes in pituitary and adrenal hormones may underlie the increase of hematopoiesis in grouped mice. Second, Fig. 2 shows that the severity of fight wounds is related to splenic EMH, suggesting tissue necrosis and infection as an underlying cause for hematopoietic response.

‖ $y = -.602\ X + 9.67$ where y = spleen weight (mg)/B.W. g, X = Tesh's weight (mg)/B.W. g. Differences in X accounted for a significant ($.010 < p < .025$ proportion of the variation of y.

The negative linear relationship between testis weight and splenic weight in subordinant grouped mice suggests that some physiological change occurring as a result of grouping acted to decrease gonadotrophin secretion in proportion to the extent it stimulated hematopoiesis.

Summary. The increased splenic weight seen in subordinant grouped male brown mice was due to increased splenic extramedullary hematopoiesis (EMH) involving RBC, WBC, and megakaryocytes. Dominant animals showed no increase in splenic weight nor increased EMH. There was a significant positive correlation between splenic weight and splenic EMH graded on an arbitrary scale. A significant positive correlation between severity of skin fight wounds and splenic EMH was found. There was a significant linear relationship with negative slope between testis weight/body weight and splenic weight/body weight in subordinant grouped animals.

1. Dawson, J., *Nature,* 196, v178, 1183.

2. Christian, J. J., *Proc. Nat. Acad. Sci.,* 1961, v47, 428.

3. Brownlee, K. A., *Statistical Theory and Methodology in Science and Engineering,* John Wiley & Sons, New York. N. Y., 1960, 189.

4. Siegel, S., *Non-parametric Statistics,* McGraw-Hill, New York, 1956, 202.

5. Maximow, A. A., Bloom, W., *Textbook of Histology,* W. B. Saunders Co., Philadelphia, Pa., 1957, 267.

6. Gordon, A. S., Piliero, S. J., Landau, D., *Endocrinology,* 1951, v49, 497.

7. Simpson, M. E., Evans, E. S., Rosenberg, L. L., *ibid.,* 1959, v64, 592.

8. Quittner, H., Ward, N., Sussman, L. N., Antopal, W., *Blood,* 1951, v6, 513.

Received May 12, 1963. P.S.E.B.M., 1963, v114.