Effects of Genetics and Anesthesia Upon Granulocyte and Agranulocyte Levels in Seven Inbred Mouse Strains. (20582)

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(Introduced by C. C. Little.)

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Recent investigations of normal mouse blood(1) has shown significant genetic differences among young adults of 18 inbred strains both in total leukocytes and in proportion of granulocytes. In addition, an inverse correlation was found between mean total leukocyte count and mean proportion of granulocytes for a given strain, suggesting "that differences in genetic factors affecting production or release of lymphocytes are more prevalent or more effective than are genes affecting granulocytes." The present experiments are designed to analyze independently the numerical variations in granulocytes (largely neutrophils) and agranulocytes (largely lymphocytes) of young adults of several of these inbred strains of mice. Inasmuch as it has been suggested that differences in reactions of animals to anesthesia might affect leukocyte values, a test of the effects of nembutal anesthesia upon the level of both types of cell was also included.

Materials and methods. After thoroughly testing its applicability to mouse blood, the Randolph method for chamber enumeration of granulocytes and agranulocytes in phloxine-methylene-blue-propylene-glycol diluent(2) was utilized in these experiments. Five virgin females and 5 males, 2 to 3 months old, from seven of the most widely used of the inbred strains in the Jackson Laboratory Inbred Nucleus were used for each determination. All counts were made between 10 A.M. and 5 P.M. during August, 1952. The anesthetized animals had been injected intraperitoneally 15-20 minutes before the sample was taken with 1.5 mg nembutal in .25 cc solution. All animals were warmed in a jar under a light bulb for 10-15 minutes before samples were taken(3), and tail blood was collected after immersing the tail for 30 seconds in hot water. Two samples were taken from each animal. The mean value for each type of cell was determined for each group of animals. Analysis of variance was used to compare the variance attributable to the particular factors which can be sorted out in this experiment (strain, sex, anesthesia, and interactions among these) with the variance due to unanalyzed factors (experimental error, slight differences in nutrition or recent activity, time of day, for example) to determine whether or not the designated factors contributed significantly to the total variation. Since the groups used were small, and the tests thereby not exhaustive, only variance contributions significant at the 1% level were considered conclusive enough to be extensively discussed.

Results. Table I gives the mean values for agranulocytes and granulocytes for 10 non-anesthetized and 10 anesthetized animals from each of the strains, both with sexes separated and combined. The totals are slightly lower than earlier total leukocyte values for the same strains by the conventional acetic acid method. In general, the observed differences between strains, sexes, and conditions of anesthesia are sufficiently small so that it is impossible to determine their significance by the standard errors of individual groups of values. Agranulocyte means vary over a wider range than do granulocytes. Table II gives the results of analysis of variance of values for each type of leukocyte. Genetic differences among the inbred strains contributed very significantly to variations in both granulocytes and agranulocytes. The effects of anesthesia (in general a lowering of the count) contribute markedly to differences in granulocyte level.

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TABLE I. Mean Values (Randolph's Method) for Granulocytes and Agranulocytes of Intact and Anesthetized Animals of Each Strain Tested, with Comparison to Earlier Conventional Determinations of Total Leukocyte Counts for the Same Strains.

Strain	Sex	Agranulocytes × 10^{-3} Non-anesthetized	Agranulocytes × 10^{-3} Anesthetized	Granulocytes × 10^{-3} Non-anesthetized	Granulocytes × 10^{-3} Anesthetized	Former total leukocyte (anesth.)
						× 10^3
A/Jax	♀	5.65	6.01	1.23	.77	
	♂	5.90	3.16	1.05	.56	
	Combined	5.77	4.59	1.14	.67	8.71
AKR/Jax	♀	3.93	5.15	1.08	.84	
	♂	7.28	6.94	1.02	1.51	
	Combined	5.60	6.05	1.05	1.18	6.80
BALB/c Jax	♀	8.52	7.47	1.64	1.15	
	♂	6.92	5.26	1.56	1.28	
	Combined	7.72	6.37	1.60	1.22	8.54
C3H/Jax	♀	5.57	5.31	1.61	1.59	
	♂	5.92	2.95	2.33	1.15	
	Combined	5.75	4.13	1.97	1.37	7.34
C57BL/6Jax	♀	7.99	8.92	.87	1.13	
	♂	8.24	6.53	.82	.89	
	Combined	8.12	7.73	.84	1.01	11.43
DBA/1 Jax	♀	7.38	6.45	1.48	1.11	
	♂	8.32	7.75	1.87	1.80	
	Combined	7.85	7.10	1.67	1.45	8.60
DBA/2 Jax	♀	7.48	5.90	1.36	.75	
	♂	7.85	6.86	1.69	1.31	
	Combined	7.66	6.38	1.53	1.03	9.28
Grand mean	♀	6.65	6.46	1.32	1.05	
	♂	7.20	5.63	1.48	1.21	
	Combined	6.92	6.05	1.40	1.13	8.41

TABLE II. Results of Analysis of Variance in Numbers of Agranulocytes and Granulocytes in Mouse Blood, with Differences in Strain, Sex and Anesthesia.

Source of variation	D.F.		Mean Sq.	F-values	Level of significance
		Agranulocytes			
Total	139				
Due to anesthesia	1	26.8669	26.8669	4.9427	.05
" " sex	1	.5982	.5982	.1101	—
" " strain	6	163.3265	27.2211	5.0078	.01
Common to sex and strain	6	78.2917	13.0486	2.4005	.05
" " strain and anesthesia	6	15.1427	2.5238	.4643	—
" " sex and anesthesia	1	16.7532	16.7532	3.0821	—
" " sex, strain and anesthesia	6	17.1878	2.8646	.5270	—
Error	112	608.7965	5.4357		
		Granulocytes			
Total	139				
Due to anesthesia	1	2.5326	2.5326	12.4208	.01
" " sex	1	.8721	.8721	4.2771	.05
" " strain	6	10.7989	1.7998	8.8269	.01
Common to sex and strain	6	2.4267	.4045	1.9838	—
" " strain and anesthesia	6	2.8113	.4686	2.2982	.05
" " sex and anesthesia	1	.0015	.0015	.0074	—
" " sex, strain and anesthesia	6	2.6105	.4351	2.1339	—
Error	112	22.8375	.2039		

It should be noted that different strains may react differently to nembutal, as factors common to strain and anesthesia produced variation (significant at the 5% level) in granulocyte number. Inspection of Table I shows that the C57BL/6 animals, in contrast to all

others, gave higher ganulocyte counts under anesthesia, and the A animals showed extreme lowering of the count with nembutal.

Discussion. These results of direct chamber count of agranulocytes and granulocytes in mouse blood have confirmed earlier findings of significant differences in total leukocyte counts among inbred strains, and have proved that there are significant differences in both types of cells, answering a question left in doubt in the earlier work. In addition, these results show that the 2 cell types vary independently of each other. The rank order in non-anesthetized animals (Table I) of mean values for agranulocytes (C57BL/6 > DBA/1 > BALB/c > DBA/2 > A > C3H > AKR) is very different from that for granulocytes (C3H > DBA/1 > BALB/c > DBA/2 > A > AKR > C57BL/6. Since the cells are produced by different tissues this is not necessarily surprising.

It might be thought that the rank order of variation for granulocytes would correspond closely with that for the erythrocytes formed in the same tissue. Taking the erythrocyte values for these strains from earlier work(1), there is a certain similarity, DBA/1 and BALB/c being high in both; however, the correlation is completely spoiled by the C3H strain, which has a very high granulocyte number but the lowest of all erythrocyte means. Separation of lymphocytic from granulocytic variation has shown no more relation between number of lymphocytes (at 2-3 months) and future potentiality for lymphatic leukemia than did the previous total and differential leukocyte counts. Thus, again it must be said that "it has not been shown that an observed strain difference is one facet of a difference in a particular known pattern of physiological balance. . . . It may equally well be that there actually is no selective advantage within the total observed range . . . (and) . . . the observed strain difference(s) might be chance fixation of a particular genotype."

The significant effect of anesthesia is of particular importance to analysis of existing literature and to planning of experiments. For example, the usefulness of the earlier work on total leukocyte counts of these same strains (1) quoted in this paper is severely limited by the fact that the total leukocyte counts were taken on anesthetized animals, while the determinations of % granulocytes were on non-anesthetized animals. The possible differing reaction of different strains to nembutal should also be of importance to the planning of experiments. Further tests of strain difference in reaction of lymphocyte and neutrophil level to nembutal anesthesia, using larger numbers of animals in each group, would be a great scientific service.

These data have definitely increased knowledge of the leukocyte characteristic of this group of inbred strains, particularly in demonstrating the reality and independence of genetic variation in granulocytes from the bone-marrow and lymphocytes from the lymphoid tissue.

Summary. 1. Direct chamber counts of granulocytes and agranulocytes were made by the Randolph method in the blood of 2-3-month-old virgin females and males of 7 inbred strains, with and without nembutal anesthesia. 2. Analysis of variance demonstrated significant genetic differences among the strains in both granulocyte and agranulocyte counts. 3. Anesthesia contributes significantly to variations in granulocyte number. 4. From these data, the observed strain differences do not appear to be related to differences in other known physiological characteristics.

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