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Selection for leukocyte counts in mice*

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1. INTRODUCTION

In 1956 we discovered that mice of SM/Ckc (small body size) were leukopenic, with an average leukocyte count of 2300 per mm^3. in the peripheral blood. In ordinary inbred strains of mice leukocyte counts range from 6000 to 11,000 (Russell *et al.*, 1951). To study the genetics of leukocyte count variation, counts were made in mice from the crossbred generations of SM and LG/Ckc. The SM mice had been developed by selection for small body size by MacArthur, and the LG mice for large body size by Goodale; subsequently the author inbred both lines (Chai, 1956), the original purpose of the crosses having been for the study of inheritance in body size. The results of leukocyte counts suggested genetic influence, and it was found that 50% of the variation in the F_2 generation could be attributed to genetic effect. We have since further investigated the genetics of leukocyte count variation, and of variations in associated biological characters in mice, with directional selection for leukocyte counts starting from a hybrid mouse stock. This paper is a report of the results of selection carried through eleven generations.

Total leukocyte count as a character for genetic study is of interest for the following reasons: (1) leukocytes play an important role in the defense mechanism of an animal, and leukocyte levels undoubtedly affect fitness; (2) the number of different cell types comprising the character to be selected can be simultaneously determined; (3) leukocytes as tissue components are relatively simple histologically as compared to other characters ordinarily used in selection, such as body growth and performance involving many tissues and organs. Gowen (1943) selected for resistance to *Salmonella* organisms in mice and found that the line showing high resistance had high leukocyte counts, while the line with low resistance had low counts. In another selection experiment Weir & Schlager (1962) reported rather high heritability in leukocyte counts in mice, and some correlation with resistance to X-irradiation.

2. MATERIALS AND METHODS

The mice used to start the selection were from a hybrid stock derived by cross-breeding six inbred strains: C57BL/6J, C57BR/cdJ, A/J, BALB/cJ, LG/Ckc and

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SM/Ckc. Among the inbred strains then available at the Jackson Laboratory C57BL/6 had the highest leukocyte count (Russell *et al.*, 1951) and SM/Ckc was leukopenic, or lowest (Chai, 1957), the other strains being intermediate. None of these strains had any known constitutional pathology at the age when the leukocyte counts were taken.

The crossbreeding design was reported by Chai in 1960. We used the conventional four-way cross with the first four strains to produce the F_2 generation. F_2 mice were then randomly selected and mated with individuals of the second generation of the cross between the last two strains. From the third generation, matings of one male with three females were set and the selection of mates was again at random. This design theoretically allows 12·5% of the genome of each of the first four inbred strains, and 25% of that of the last two to be incorporated in the stock with which selection started. The fourth generation of crossbreeding was designated as zero in the present study, since it was at this generation that we began the selection of mates.

By using directional selection based on individual merit for high and low total leukocyte counts two lines of mice were established, one for high and one for low leukocyte counts, designated HLC and LLC respectively. In addition, a random-bred line derived from the same hybrid stock was maintained and designated RLC.

As a rule we set ten to twelve pair matings for the LLC and HLC lines and about twenty for the RLC line, except in the zero generation where one male was mated to three females. No consideration was given to ancestry when the individuals were chosen for mating. Single matings were set by random pairing, but we avoided setting sib-mating and in case of a sib pair being chosen, one member was replaced with a non-sib if available. As a rule the first two litters were used from each mating. Since there was a high rate of sterility at generation 6 of the LLC line, replacements were made for sterile matings where possible. We constantly reserved selected animals as replacements for poor matings, but since matings were set after the blood count, the reserved mice were usually over 6 months old when the original matings proved poor or sterile. In certain generations, therefore, the replacements were too old to be used, and we were unable to produce sufficient numbers of animals.

Blood samples were taken at intervals of 3 to 4 weeks, at between 1.00 and 2.00 p.m., from the lateral tail vein of mice 60 to 90 days old. One sample was obtained from each mouse in generations 0–4, and two from each in subsequent generations. Before blood was taken the mice were placed in a battery jar and heated for approximately 10 min. under a 100 W. light bulb to stimulate peripheral blood circulation. Each tail was then washed with soap and warm water and wiped thoroughly dry. A cross incision was made on the tail vein with a razor blade, and immediately after the appearance of the first two drops of blood a sample for the total leukocyte count was taken with a leukocyte-counting pipette. A small drop of blood was then laid on a microscope slide to provide a smear for differential counts, two smears being made for each mouse. The pipette was filled with 3% glacial acetic acid to make a 10-times dilution and shaken vigorously, first by hand and then

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on a mechanical blood pipette shaker, until counting time. These operations had to be performed quickly to avoid coagulation of the blood.

Two blood-counting chambers of a hematocytometer were filled with each diluted blood sample, and after allowing about 2 min. for sedimentation total leukocyte counts were made.

The blood smears were dried under a light bulb, then fixed in methyl alcohol and stained with Hemal Blood Film stain produced by Scientific Products (instructions given by the Company). One hundred cells were read in each slide and the percentages of neutrophils, eosinophils, lymphocytes, and monocytes recorded. The actual count for each cell type was obtained by multiplying its percentage by the total cell count.

Purina Laboratory Chow was used through the third generation and Old Guilford thereafter, the latter being apparently more palatable, with more fat content. During the period of the experiment the leukocyte counts operation was performed with the help of three successive persons. To minimize technical variation we were careful to keep the procedures consistent, and to see that each assistant had completely mastered the technic before taking over. Fortunately, two of the three assistants were involved in only the first two generations, while the third had charge of leukocyte counts during the remainder of the experiment. If inconsistencies did occur, therefore, they should not affect comparisons between the later generations.

Coat color and 60-day body weight were recorded for each mouse as were the percentages of sterility, litter size, still births, and number weaned for each mating. Mice in the later generations of each line were X-irradiated with three different doses and the percentages of mortality after irradiation recorded. Leukocyte counts were made in the last generation.

Table 1. *Average state of inbreeding* (ΔF), *and inbreeding coefficients* (F) *at the eleventh generation of each line*

	ΔF	F
HLC	0·026	0·23
RLC	0·014	0·16
LLC	0·042	0·35

3. RESULTS

(i) *Inbreeding increment*

In a closed population the smaller the number of matings the greater the inbreeding coefficient. From the number of effective breeders in the different generations we computed the average increment of inbreeding coefficient (ΔF) per generation and the inbreeding coefficient (F) in the last generation according to the formulas given in Falconer (1961). These formulas take into consideration neither the increase of inbreeding due to selection in the HLC and LLC lines nor the reduction

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of inbreeding due to our practice of trying not to set sib-mating. The estimates of ΔF and those of F at the eleventh generation are given in Table 1. The rate of increment of the inbreeding coefficient was greater in the LLC than in the HLC line, and greater in HLC than in RLC. Actually the increment was about the same between the LLC and HLC lines up to the sixth generation, where a scarcity of LLC breeders was reflected in a large increment for that line.

(ii) *Production records*

Average litter size, still births and average number of mice weaned per litter were computed for each generation of each line, as were the averages for all the generations (Table 2). The average number weaned would have been greater if we had not kept a maximum limit of eight newborn in each litter. Although there appear to be no large differences in the averages between the three lines, litter size

Table 2. *Average litter size and average number of still births per litter for all generations of each line*

	Litter size		
	Birth	Weaning	Still birth
HLC	7·2	6·6	0·06
RLC	7·0	6·6	0·06
LLC	6·5	6·3	0·19

and number weaned were smaller, and still births higher, in the LLC than in the HLC and RLC lines. On the average the LLC line produced and weaned 0·3 mice less, and had 0·13 still births more per litter than the HLC line; and HLC mice produced 0·8 mice less than the RLC, but had the same number weaned. When all the generations were pooled, the LLC line showed much greater sterility (33%) than the HLC (7%) and the RLC (9%) lines.

(iii) *Body weight*

The 60-day body weight was taken for each mouse beginning with the first generation in each line, the means and standard deviations for females and males of each generation being computed separately. The means against generations in each line were plotted in Fig. 1. The mean body weights were higher than those of most inbred strains because one-quarter of the LG/Ckc (large body size) genome was contained in the original hybrid stock. A gradual increase in body weight appeared in the selected lines with the advance of selection, but was very slight in the RLC line. At the eleventh generation the differences in the means for the same sex between the RLC and either one of the other two lines were significant. The standard deviations, not given here, were rather similar in magnitude between the three lines in the first two generations, but from the third onward practically

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all those of the LLC line were higher than those of the other two. Many of these differences are highly significant. Although the means of the LLC were higher than those of the RLC line, they were not large enough to account for the high standard deviations.

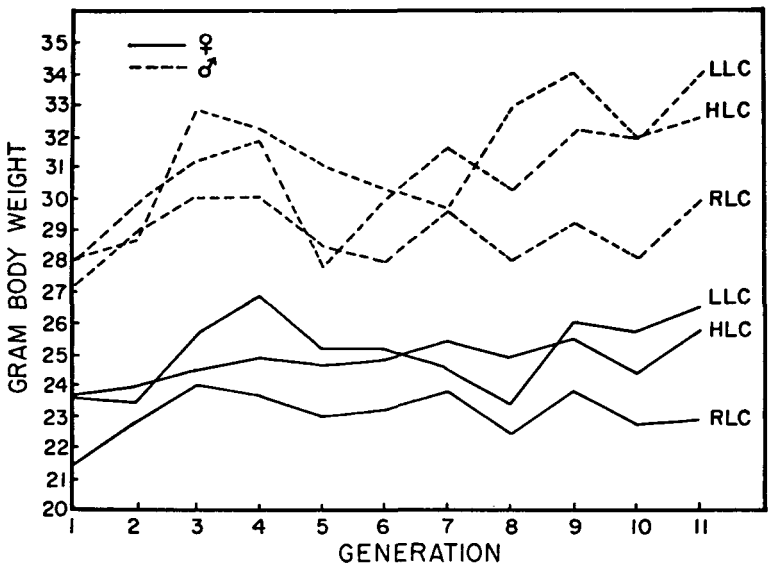

Fig. 1. Plots of mean 60-day body weight (grams) for mice of each generation of each line.

(iv) *Coat color*

Coat color genes carried in the stock at the beginning of selection were A^+, A^w, *a*, *B*, *b*, *C* and *c*, and coat colors were recorded for each mouse except in the first two generations of each line. In the eleventh generation of the HLC line the percentages of animals having the different coat colors were 5·6 *cc*, 2·2 A^+-*B*- and 92·1 *aaB*-; A^w- and *bb* animals disappeared from this line after the fifth generation. In the eleventh generation of the LLC line we recorded percentages of 30·4 *cc*, 53·2 A^+-*B*- and 16·3 *aaB*-. The frequencies of A^w mice decreased with advancing generations and disappeared in the last. *bb* animals disappeared after the seventh generation, and these alleles have probably dropped out in this line. In the RLC line, phenotypes for the different combinations of alleles as carried at the beginning of selection were still found in the eleventh generation, but there was a trend toward decrease in the frequency of the A^w and *B* alleles.

The frequency of *cc* animals in the different generations of each line is shown in Table 3. In the HLC line it was low at the beginning and remained low, whereas in the LLC and RLC lines it varied from 12·0 to 50·6 and from 10·4 to 24·8 respectively. The change noted in gene frequencies in the random line was probably a consequence of random selection of mates, or of a drift under our management.

No significant difference in leukocyte counts in relation to coat color was found by analysis of the records in the early generations of the RLC line.

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Table 3. *Percentage of albino* (cc) *mice in each generation of each line*

Gen.	HLC	RLC	LLC
2		14·6	
3	5·5	20·4	25·6
4	9·8	12·9	32·4
5	7·0	17·4	19·3
6	0	23·8	12·0
7	13·3	22·8	39·2
8	16·0	23·7	56·5
9	20·1	24·8	38·9
10	0	24·7	50·6
11	5·6	10·4	30·4

(v) *Leukocyte counts*

A histogram was made for the distribution of leukocyte counts for the mice in each generation of each line, and their general pattern examined. Many appeared symmetrical, although some were skewed to the right. The degree of dispersion varied among generations and lines, but there was no clear-cut bimodal type of distribution. Tests for normality of the distributions of leukocyte counts in each of four generations of each line showed the logarithmic scale to be superior to the arithmetic; the latter, however, had the advantage of being simpler and more comprehensible, so both scales were used in the following analysis.

The means and standard deviations of the total leukocyte counts were computed for each generation of all three lines, one diagram plotting the means against generations for each line on an arithmetic scale (Fig. 2*a*) and one the deviation of each mean of the HLC and LLC lines from that of RLC on a logarithmic scale (Fig. 2*b*). It is clear that the responses to directional selection were asymmetrical, more so on the arithmetic than on the logarithmic scale. Fluctuation in responses to selection were reduced when the means of the HLC and LLC lines were plotted as deviations from those of RLC. In a comparison of the means between females and males in each line, those of females showed higher in the HLC line, but not in LLC or RLC.

In order to examine the general trend of response in the counts of each type of cell we plotted the means for each cell type of the HLC and LLC lines as deviations from those of RLC by logarithm, as this method apparently removed some fluctuations of response (Figs. 3 and 4).

Beginning from the third generation, neutrophil counts (Fig. 3*a*) for LLC mice were below those of RLC and remained so through the later generations. In the HLC line, there was a gradual increase up to the eighth generation; thereafter the counts dropped and leveled off. The mean counts for males were greater than those for females in the late generations of the HLC line.

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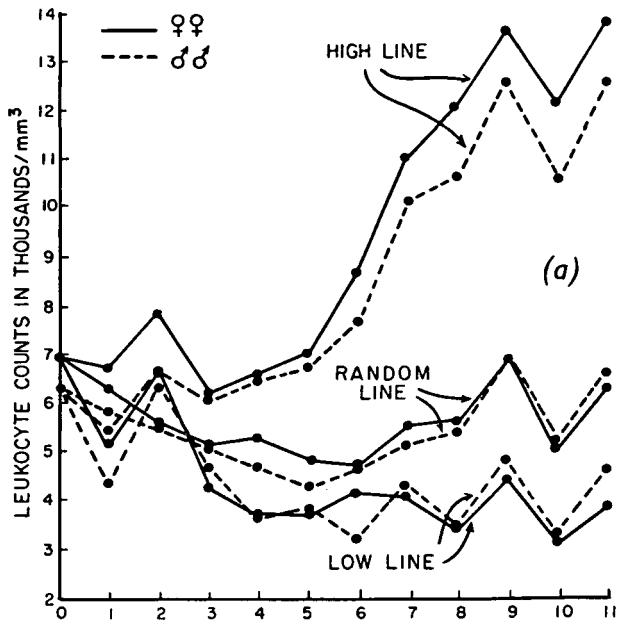

Fig. 2*a*. Graphs of the mean arithmetic total leukocyte count in each generation of each line.

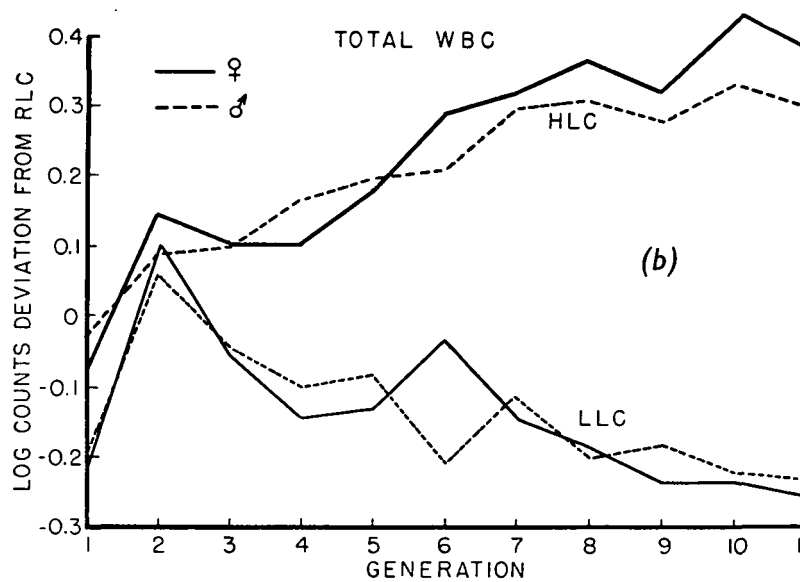

Fig. 2*b*. Graphs of the mean logarithmic total leukocyte counts of the HLC and LLC lines as deviation from those of RLC.

The general trends for the eosinophil counts (Fig. 3*b*) for the three lines appeared similar to those of the neutrophils, though there were much larger fluctuations among the generations, especially in the LLC line, probably because the eosinophil counts were so small. The means for males were greater than those for females in

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the later generations of the LLC line. The rather low counts for females in the first generations of both the high and low lines can be considered due to sampling and possibly technical errors.

The distributions of the lymphocytes closely followed the pattern of those of the total leukocyte counts (Fig. 4*a*) as was to be expected since lymphocytes are predominant in the total leukocyte count for mouse blood. The wide separation

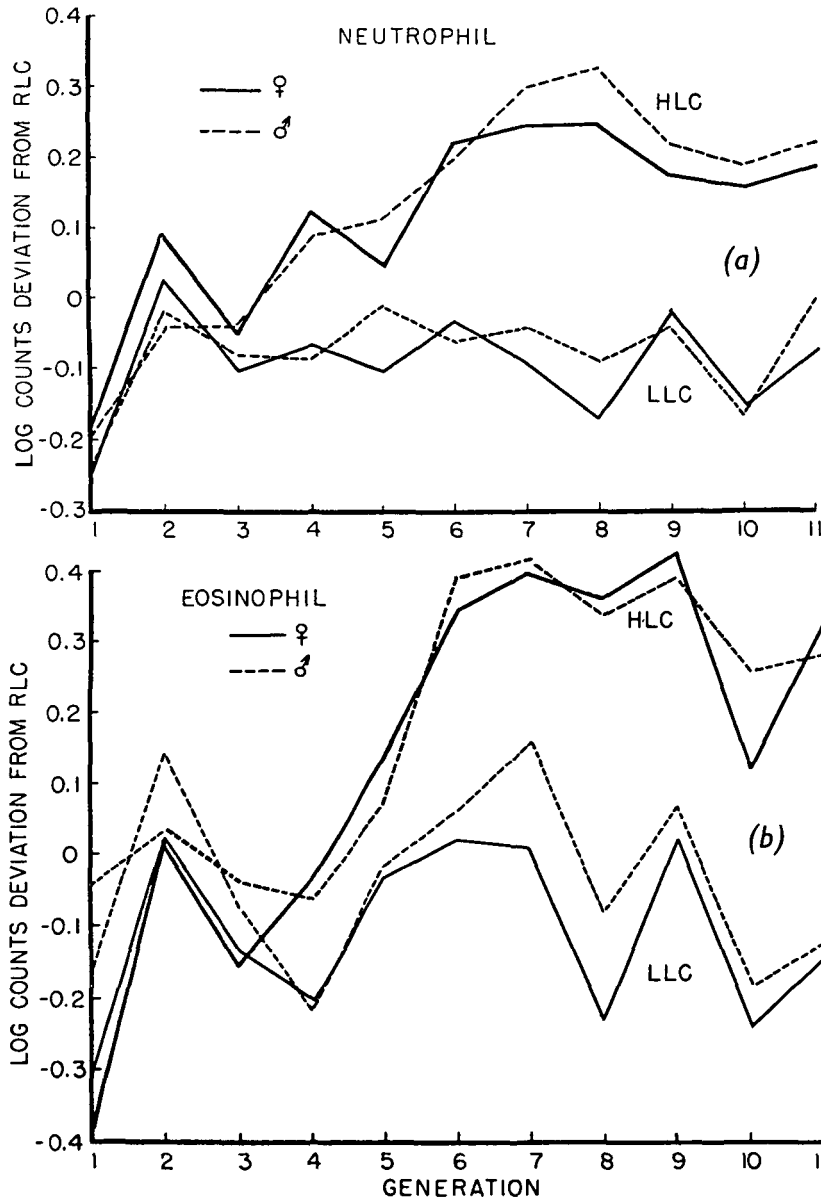

Fig. 3. Graphs of the mean logarithmic counts of individual cell types in each generation of the HLC and LLC lines as deviations from those of RLC

a. Neutrophils

b. Eosinophils

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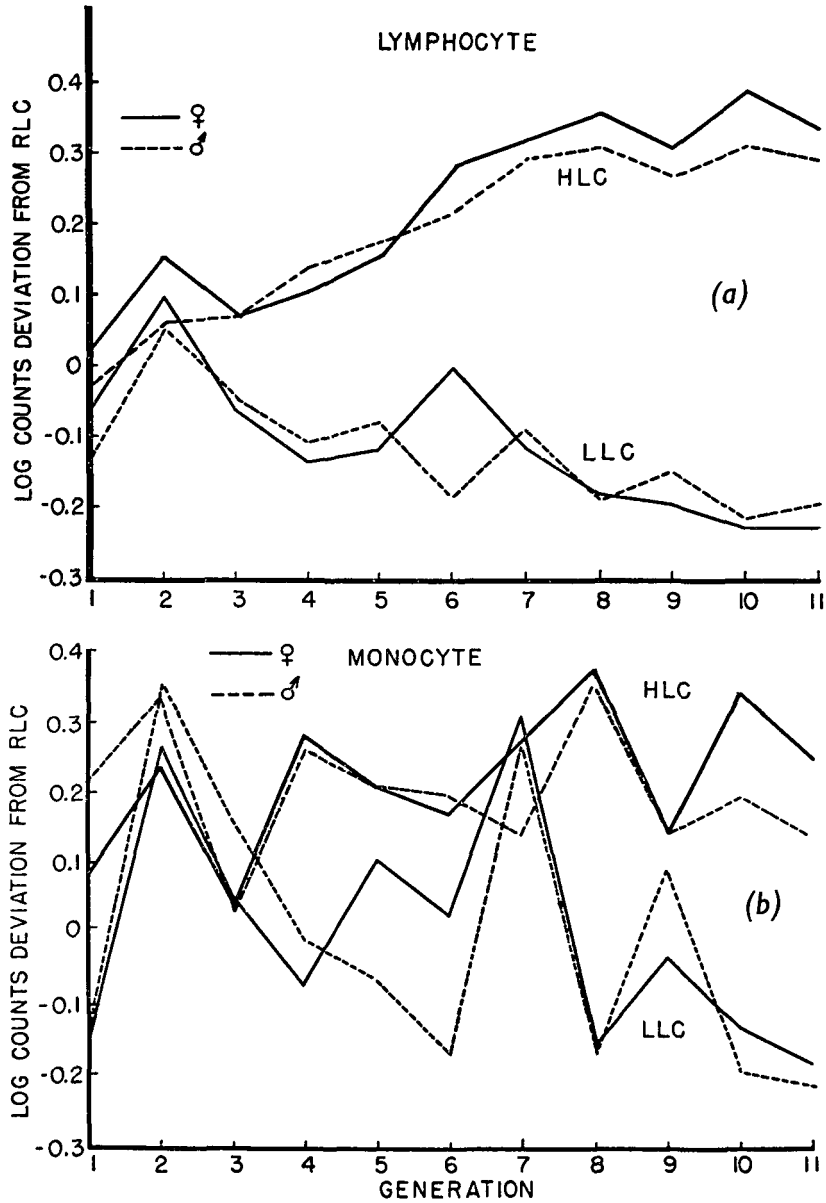

Fig. 4. Graphs of the mean logarithmic counts of individual cell types in each generation of the HLC and LLC lines as deviations from those of RLC
a. Lymphocytes
b. Monocytes

of the HLC and LLC lines in the mean level of lymphocyte counts, and the general pattern of distributions closely resembling that for the total counts (Fig. 2*b*), were also to be expected. The responses fluctuated less than those of any other cell types and were slightly asymmetrical. Beginning from the sixth generation of the HLC line, the means for females were consistently higher than those for males, clearly

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indicating that such sexual differences were the main contributing factors for sexual differences in the total leukocyte counts.

The responses of monocyte counts to selection were different from those of any of the other cell types (Fig. 4*b*). The generation means of the HLC line were slightly higher than those of RLC and remained at a similar level through all the generations. There was a tendency to decrease with the advance of selection in the LLC line. Among the four cell types the monocyte is rare, and sometimes difficult to distinguish from the lymphocyte, accounting possibly for the large fluctuations in the responses. Therefore, much emphasis on the distribution patterns is not profitable.

The means for the total and for the individual cell-type counts in each generation of each line are too comprehensive to present here, but for purposes of comparison between lines and examination of the advance by selection, the averaged means and standard deviations for generations 0–2 and 9–11 are given in Table 4. Incidentally, these values may be of assistance in the study of mouse hematology, since statistics for differential leukocyte counts in mouse blood are lacking.

(vi) *Percentages of different types of leukocytes*

We computed the mean percentages and standard deviations for the different cell types, and examined the general trend of change among the generations in each line and the differences between lines. The percentages of the two major cell types, lymphocytes and neutrophils, apparently influenced by selection, showed significant changes. In the first three or four generations no noticeable differences occurred between the three lines; the levels of percentage were rather high for lymphocytes and low for neutrophils, with a general tendency toward gradual decrease in the percentage of lymphocytes, and increase in that of neutrophils through the third or fourth generation. Thereafter, the three lines separated. The decrease in lymphocyte percentage and increase in neutrophil percentage continued in the LLC line, whereas in the HLC line the reverse became true. Thus the divergence between the two selected lines with respect to the percentage of these two cell types widened with the advance of generations. The percentage levels for both cell types remained intermediate in the RLC line. There was also a rather consistent sex difference in all these lines, the females having a larger percentage of lymphocytes, and a smaller percentage of neutrophils, than males.

The two minor cell types, eosinophils and monocytes, showed larger percentage variations within than between lines, perhaps because of sampling errors due to their scarcity. However, more generations showed higher percentages of both eosinophils and monocytes in the LLC than in the HLC line. Averages for generations 0–2 and 9–11 are presented in Table 5.

(vii) *Heritability*

Selection differentials and responses of total leukocyte counts were computed logarithmically for each of the selected lines. Instead of comparing the means of

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Table 4. *Averaged means and standard deviations of total and differential leukocyte counts for the early* (0–2) *and late* (9–11) *generations of each line* (*multiplying each value by* 20 *gives count per mm*^{3})

Sex	Gen.	Line	No. of mice	Total		Neutrophils		Eosinophils		Lymphocytes		Monocytes	
				Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
♀	0–2	HLC	549	352·8	131·5	46·1	20·1	9·7	6·4	288·7	120·0	7·8	9·6
		RLC	666	329·4	126·5	44·7	17·9	8·5	5·2	270·2	119·2	6·1	4·3
		LLC	556	338·7	122·4	45·5	21·3	9·2	5·5	275·6	102·4	7·7	7·6
	9–11	HLC	184	656·7	155·2	60·4	22·3	15·8	10·0	571·7	146·6	9·1	8·0
		RLC	153	308·1	93·9	40·7	19·1	7·3	5·4	255·4	80·4	4·5	3·6
		LLC	129	265·8	72·3	34·3	18·5	5·6	4·1	147·8	58·9	3·6	3·7
♂	0–2	HLC	524	317·1	135·6	52·5	27·1	9·2	6·6	246·9	109·1	7·5	8·8
		RLC	644	307·0	126·1	53·7	20·4	8·3	4·6	238·8	88·3	5·6	3·9
		LLC	531	307·6	128·5	52·0	21·7	9·7	6·2	238·3	95·5	7·3	7·7
	9–11	HLC	188	601·0	150·7	75·0	32·2	15·5	9·7	503·0	134·2	7·6	6·7
		RLC	159	311·0	85·8	45·4	18·7	7·4	5·2	254·5	75·2	4·4	3·5
		LLC	134	212·2	88·2	39·8	19·0	6·2	4·2	162·7	72·6	3·4	2·6

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Table 5. *Averaged means and standard deviations of percentages of each cell type in the early* (0–2) *and late* (9–11) *generations of each line*

	Neutrophil			Eosinophil			Lymphocytes			Monocytes		
♀	HLC	LLC	RLC	HLC	LLC	RLC	HLC	LLC	RLC	HLC	LLC	RLC
Gen. 0–2												
Mean	12·01	12·41	14·08	1·62	1·87	1·46	84·22	83·43	83·82	2·18	2·36	0·65
s.d.	3·74	4·23	3·67	1·26	1·51	0·95	4·94	5·16	4·04	2·33	1·98	0·71
Gen. 9–11												
Mean	9·42	18·15	13·31	2·45	2·91	2·46	86·77	77·19	82·71	1·36	1·84	1·52
s.d.	3·32	6·61	4·79	1·59	1·70	1·62	4·01	7·25	5·13	1·10	1·30	1·10
♂												
Gen. 0–2												
Mean	13·89	14·26	17·33	1·84	2·06	1·61	81·95	80·98	80·46	2·29	2·49	0·63
s.d.	6·52	5·41	5·94	1·89	1·58	1·10	7·84	6·25	6·30	2·11	1·76	0·69
Gen. 9–11												
Mean	12·53	19·07	14·70	2·60	2·96	2·37	83·60	76·41	81·52	1·26	1·56	1·41
s.d.	5·18	7·36	5·69	1·56	1·73	1·49	5·57	8·31	6·22	1·14	1·14	1·09

the previous generations, the deviations of the means of each generation of the selected lines from that of the RLC line were used to measure response, since in many generations seasonal fluctuations were large. A regression coefficient was computed for each selected line. Using the average increment based on regression divided by the average selection differential for estimating heritability (h^2), we obtained h^2 estimates of 0·21 and 0·15 in the HLC and LLC lines respectively.

Heritability was also estimated, using the half-sib relationships in the zero generation. The mean square for sires, and for dams within sires, was computed according to the standard method as follows:

Sources of variation	*Estimates of mean squares*
Between sires	$\sigma_w^2 + k_2\,\sigma_d^2 + k_3\,\sigma_s^2$
Between dams within sires	$\sigma_w^2 + k_1\,\sigma_d^2$
Within dams	σ_w^2

Here σ_w^2 is the variance between mice within dam, σ_d^2 the variance between means of the dam families, σ_s^2 the variance between means of the sire families, and k_1, k_2, and k_3 are the harmonic means of the numbers of mice in the respective groups.

The portions of the different genetic variances contained in the sire and dam components can be stated as follows (Kempthorne, 1957):

$$\sigma_s^2 = \tfrac{1}{4}\sigma_A^2 + \tfrac{1}{16}\sigma_{AA}^2 + \tfrac{1}{64}\sigma_{AAA}^2 + \cdots$$
$$\sigma_d^2 = \tfrac{1}{4}\sigma_A^2 + \tfrac{1}{4}\sigma_D^2 + \tfrac{15}{16}\sigma_{AA}^2 + \tfrac{1}{8}\sigma_{AD}^2 + \tfrac{1}{16}\sigma_{DD}^2 + \tfrac{7}{64}\sigma_{AAA}^2 + \cdots$$
$$\sigma_w^2 = \sigma_E^2 + \text{remaining portions of the genetic variances.}$$

We used a total of 381 female and 376 male mice in the analysis. Each group contained ten sire families, and each sire was mated with three dams, except one which was mated with one dam only. Because of differences in the leukocyte counts between sexes, the analysis was performed separately for each sex. The computed k values according to the method outlined in Kempthorne (1957) were 13·5, 13·4, and 37·4 for k_1, k_2, and k_3 respectively in the female group, and 13·3, 13·2, and 36·8 in the male group, showing that distribution of females and males was approximately the same in either a dam or a sire family. The two sets of variance components resulting from the analysis are given in Table 6, one on an arithmetic and the other on a logarithmic scale. Since the sex ratio is close to one, a straight average for the sexes is taken in each variance.

The difference between σ_d^2 and σ_s^2 was large according to both computations, but larger in the logarithmic, where σ_d^2 was twice or more as great as σ_s^2 while on the arithmetic scale it was only 1·5 times as great. Accordingly, the differences between the two variances may be attributed to $\frac{1}{4}\sigma_D^2$ plus the relevant portions of the different orders of interactions, and to possible maternal and cage effects.

We computed the heritabilities (h^2) of total leukocyte counts using the above estimated variances (Table 6) and the following formulas:

$$\frac{4\sigma_s^2}{\sigma_s^2+\sigma_d^2+\sigma_w^2}, \quad \frac{4\sigma_d^2}{\sigma_s^2+\sigma_d^2+\sigma_w^2} \quad \text{and} \quad \frac{2(\sigma_s^2+\sigma_d^2)}{\sigma_s^2+\sigma_d^2+\sigma_w^2}.$$

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Table 6. *Variances of total leukocyte counts in the zero generation attributed to sire (σ_s^2), dam (σ_d^2), and individuals within dam (σ_w^2), computed on arithmetic and logarithmic scales, and heritability estimates based on the variances*

	Arithmetic			Logarithmic		
	Variance					
Sex	σ_s^2	σ_d^2	σ_w^2	σ_s^2	σ_d^2	σ_w^2
♀	948·0	1381·8	13860·1	0·00093	0·00245	0·02120
♂	903·7	1449·1	16719·4	0·00116	0·00252	0·02380
Av.	925·9	1415·5	15289·9	0·00105	0·00249	0·00250

	Heritability					
	Sire	Dam	Sire + dam	Sire	Dam	Sire + dam
♀	0·234	0·341	0·288	0·151	0·400	0·275
♂	0·190	0·304	0·247	0·169	0·367	0·268
Av.	0·212	0·323	0·267	0·160	0·384	0·272

As for computing the average variances, an average h^2 was taken for both sexes. As expected, h^2 was greatest when the estimate was based on the dam component of variance, least on the sire component, and intermediate on both sire and dam components. The differences were greater on the logarithmic than on the arithmetic scale. It is interesting that if the two scales are compared with respect to the estimate, an increase of h^2 based on σ_d^2 on the logarithmic scale of leukocyte counts appears to be at the expense of h^2 based on σ_s^2; thus the similar values obtained on both scales from an estimate based on both sire and dam components reflect the effects of scale in the analysis.

(viii) *X-irradiation*

Doses of 650 r., 750 r., and 850 r. of X-irradiation, each delivered at a rate of 83 r. per minute, were applied to mice of each of the three lines. Ten mice were used for each dose, or a total of thirty in each generation of each line. X-irradiation was started from generation 6 in the LLC and HLC lines and from generation 7 in the RLC line. (No mice were irradiated in the seventh and tenth generations of the LLC line, due to a shortage of mice.) The rate of mortality within one month after irradiation was tabulated according to dose, generation and line, and averaged for all generations of each line (Table 7).

The mortality rate showed rather large variation among generations within the HLC and RLC lines, especially in the group receiving 850 r. The mortality rate was higher in the LLC than in the HLC and RLC lines for each generation in the 650 r. and 750 r. dose groups, and also in the average mortality rate of each line for all dose groups. Between the RLC and HLC lines the differences in mortality

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Table 7. *Number of mice dead by X-irradiation out of* 10 *in each generation of each line*

Line	HLC			RLC			LLC		
Dose (r.)	650	750	850	650	750	850	650	750	850
Gen. 6	1	4	9	—	—	—	5	8	8
7	1	2	1	0	2	4	—	—	—
8	3	5	9	4	5	6	4	9	8
9	1	3	1	2	3	9	4	7	9
10	3	3	10	0	6	6	—	—	—
11	4	6	6	0	0	10	8	10	9
Av.	2·2	3·8	6·0	1·2	3·2	7·0	5·2	8·5	8·5

rate in the different generations were inconsistent. When the mortality rate for each dose group was pooled for all the generations of each line the difference between the 650 r. group and either one of the other two groups was significant. But between the 750 r. and 850 r. groups the differences in the HLC and RLC lines, though large, were not significant, and there was no difference in the LLC line. The average mortality rates for all doses and generations were 74·2, 38·0, and 40·0 for LLC, RLC and HLC respectively. The differences in these values between LLC and either one of the other two lines were significant ($P < 0{\cdot}01$). No consistent significant differences in the total leukocyte count made within each generation in each line before irradiation were found between the mice who died and those who survived the irradiation. However, leukocyte counts made in one generation for all three lines on the fifth day after irradiation (the day on which the leukocyte count is generally lowest) were lower for LLC mice than for those of the HLC and RLC lines.

4. DISCUSSION

Eleven generations of selective breeding for total leukocyte counts produced two lines of mice which differ from each other not only in leukocyte counts but also in reproductive performance, body weight variation, coat color, resistance to X-irradiation and inbreeding coefficient.

Beginning with the third generation, increased variation in body weight occurred in the LLC line, indicating decreased fitness. Additional indications of such a decrease were a sudden drop in reproductive performance, with a high percentage of sterility in the sixth generation, and greater mortality from X-irradiation in LLC mice than in those of the other two lines. Up to the seventh generation the increment of inbreeding in the LLC line was about the same as that in the HLC line, suggesting that low leukocyte counts may be the main factor causing decrease in fitness in the early generations. From the eighth generation LLC mice were further hampered by a rapid increase in rate of inbreeding—an additional factor affecting fitness.

These results of X-irradiation on LLC mice mentioned above suggest that the

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leukocyte level itself does not account directly for differences in radiation resistance in these mice. Neither is it clear how the leukocyte level affects their reproductive performance. However, some direct or indirect underlying physiologic and genetic mechanisms must be in operation in view of the differences in these characters between the LLC and the other two lines.

The lack of response to selection in the first two generations, similar to that reported by Weir & Schlager (1962), may be associated with the breeding history of the hybrid stock from which the selection started; since the stock used in the present experiment was produced by intercrossing six inbred strains of mice to the fourth generation of crossbreeding, there may not have been a long enough succession of generations to allow for recombination and segregation of closely linked genes in the parental strains. In the third and fourth generations of selection there was a large response in the LLC line and only slight response in the HLC line; but in the later generations that situation was reversed. These results may be interpreted as indicating that a small number of genes with relatively large individual effect on the leukocyte level were involved, and also that some genes with minus effect may have been dominant over those with plus effect, since dominance of a favored gene aids selection when the gene is rare, but hinders it when the gene is more abundant than the undesired recessive (Wright, 1931).

With respect to the different types of leukocyte cells, selection for total counts may be considered simultaneous for counts of each type, with a differential preference or pressure corresponding to their percentages of the total counts. Thus the result of selection for the total leukocyte counts should be largest for the lymphocytes, next largest for the neutrophils and smallest for the eosinophils and monocytes, and in general the results we obtained conformed to this pattern.

Coefficients of correlation between the counts of the different cell types were computed in the third, sixth and ninth generations of each line, and the resulting averages are given in Table 8. The coefficients in most cases ranged around 0·30 or above, except that many of those for the monocytes and the others are smaller.

Table 8. *Average correlation coefficients between counts of different cell types for generations* 3, 6, *and* 9 *of each line. Coefficients in the upper right half of the matrix are for males and those in the lower left half are for females*

	Neutrophil	Eosinophil	Lymphocyte	Monocyte
Neutrophil		0·285	0·386	0·241
Eosinophil	0·309		0·301	0·220
Lymphocyte	0·408	0·331		0·216
Monocyte	0·256	0·179	0·328	

The percentage changes in the different cell types in the selected lines were as expected. Lacking complete correlation between the counts of the different cell types, a large numerical increase in the lymphocytes would cause percentage decrease in the other cell types, as in the HLC lines, and the converse would also be true as in the LLC line.

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The problem of control populations for artificial selection programs was discussed by Bray *et al.* (1962). A control population ideal in the strict sense cannot be maintained. Any population, with or without artificial selection, is continuously subject to natural selection, drift and mutation, and the interaction of these factors with artificial selection is unknown. The purpose of maintaining the RLC line in our present study was mainly to observe evolutionary changes and the variability of polygenic characters of highly adaptive significance, and to obtain from it crude information regarding seasonal influence—for example, the fact that seasonal fluctuations occurring in the ninth and tenth generations were all in the same direction for all three lines.

An interesting result of this study lies in the sexual difference apparent in the counts for individual cell types. In most generations of the HLC line females had lower neutrophil but higher lymphocyte counts than males. In the LLC line, females had lower eosinophil counts than males. Sexual differences may be correlated with physiological differences, such as qualitative or quantitative variations between male and female hormones, which affect the production or destruction of certain types of leukocytes; or behavioral differences affecting endocrine activity. There must be some genetic basis for these differences, but we prefer to defer our interpretation until more data have been accumulated.

In long-term directional selection in laboratory animals the selected lines eventually produce populations carrying selected genes on a special genetic background with a high percentage of homozygosity. As a consequence, additive genetic variance decreases and, at the same time, environmental variances may increase. The additive effect of a gene in an early generation may not be at all the same as it is in a later one. In such selected populations estimates of heritability based on selection differential and response would be different among the generations, and meaningless. Using the regression of selection response for making such estimates is a crude procedure, but it does eliminate much of the large bias caused by environmental fluctuations and interactions. The differences in the estimates of h^2 based on sire and dam components of variance were possibly due to non-additive genetic vairation and environment common to sibs. Nevertheless, the two sets of estimates, based on different generations of mice and using different genetic relationships, agree fairly well with each other and also with the estimates of Weir & Schlager (1962).

SUMMARY

By directional selection for total leukocyte counts from a hybrid mouse stock we have gradually established two lines of mice, LLC (Low Leukocyte Count) and HLC (High Leukocyte Count), which differ both in total and in differential leukocyte counts. A randombred line (RLC) is also being concurrently maintained. Other variations between these lines of mice are in body weight, in the frequencies of coat color genes, reproductive performance, and resistance to X-irradiation. The LLC line was comparatively low in the latter two physiological parameters, and high in variation of body weight.

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Responses to selection for high and low leukocyte counts were asymmetrical. In the first two generations, responses were irregular; thereafter they were large in the low line (LLC) for two or three generations and then became small in comparison with those of the high line (HLC). At eleven generations of selection, the mean leukocyte count of HLC is about three times that of LLC. Responses of the different cell types were proportional to their individual percentages of the total counts. There were sexual differences in the counts of total and individual cell types. Selection for total leukocyte counts affected the proportions of the individual cell types. Heritability estimates based on selection differential and response and on sib relationships yielded values ranging from 0·15 to 0·39.

Variations among generations and mouse lines resulting from selection are discussed.

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