SELECTION FOR LEUCOCYTE COUNT IN THE HOUSE MOUSE AND SOME PHYSIOLOGICAL EFFECTS¹

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REASONS for applying artificial selection to non economic species are (1) to produce new stocks or strains for some specific purpose and (2) to throw new light on the theory of selection. Our selection experiment started out with the first objective, but will be described with emphasis on the second. The reason for this is that the two strains of mice, LCH and LCL, derived by two-way selection for total leucocyte count have been lost. Selection was continuous for 20 generations, but with no progress beyond the 13th. This was not surprising because full-sib matings were employed exclusively except for several nonsib matings in the early generations. Selections were based on family and individual merit within a closed system. By generation 13 the mean counts were: LCH 17,500; LCL 4,000 cells per cubic millimeter. Had the original purpose been to study the effects of selection, intense inbreeding would have been avoided; otherwise there would have been no change in the experimental design.

Choice of a character to be selected may be determined solely by availability of materials and ease of measurement (body weight, tail length, amount of white spotting), by promise of practical applications (efficiency of food utilization, susceptibility to dental caries, milk production, resistance to disease) or a combination of the two. Selection for total leucocyte count falls in the third category.

The technique for counting blood cells is a standard one, and by making all counts when the mice were 30 days of age, a major source of nongenetic variation was minimized. The application to problems of medical interest rests on the known functions of leucocytes in immunity reactions. Since inbred lines of mice differ in total leucocyte count (e.g. GOWEN and CALHOUN 1943) it seemed reasonable to suspect that response to selection would be obtained so long as the initial stock was of hybrid origin. The outbred stock designated T and developed by MACARTHUR (1944) from crossing seven laboratory strains not previously employed in disease resistance studies seemed ideally suited to the purpose. Inoculation of mice not needed for matings, using a standard dose of 200,000 live organisms of *Salmonella typhimurium*, provided the appropriate data to determine the influence of leucocyte count on resistance to infection. The resistance level of the T mice with survival ranging from 40 to 80 percent corresponds with

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the E and Z strains of GOWEN and CALHOUN (1943). These strains were intermediate between the resistant S and RI and susceptible Ba and L (BALB/GW and LGW). Results of the tests for resistance of LCH and LCL mice to disease and to X-irradiation may be summarized as follows: (1) Leucocyte numbers *per se* confer resistance to irradiation, but not to artificial infection with mouse typhoid. (2) The LCH and LCL strains were both more susceptible to mouse typhoid than the outbred T. A brief account of the experiments through ten generations of selection including disease resistance studies has been published (WEIR, COOPER and CLARK 1953).

MATERIALS AND METHODS

The outbred line, T, was the result of random mating the 26 F₁ and all subsequent generations from crosses between "standard Laboratory strains" described as follows: black and tan (*a'*) × "cinnamon spotted" (*bs*) (9), albino (*c*) × dilute brown (*d b*) (3), "silver chocolate" (*si b a*) × *p se d b a* (6) and black and tan × wild type (8). In each case the female parent is listed first and the number of F₁ young is shown in parentheses (from MACARTHUR 1944). In a later, less detailed paper (MACARTHUR 1949), the number of inbred strains is given as six. Our designation, T, stands for Toronto, the stock having been obtained from DR. L. BUTLER of the University of Toronto. In our hands the "strain" from the outset, and continuously (without appreciable changes in gene frequencies) has been segregating for black and tan (*a'*), nonagouti (*a*), albino (*c*), brown (*b*), leaden (*ln*) and the wild-type alleles of the respective mutants.

The dilution factor has been determined by genetic tests, in our laboratory and elsewhere, to be due to *ln* and not *d*. It may be that the mice described as "silver chocolate" and designated by MACARTHUR as "*sv b a*," may have been leaden chocolate, and that the dilute allele contributed by "the familiar long-inbred 'dilute brown' (*dba*)" and the "*p se d b a*" did not persist. In our laboratory no mice with silver, piebald, pink eye, dilute, or short ear phenotypes were included in the original 22 females and 15 males obtained in 1949, nor have any appeared since. There were a few pygmy (*pg*) mice in early generations, but these were eliminated. Although the details concerning the origin of our T line of mice are somewhat confused, the suitability of the mice for the purpose intended is beyond dispute. The advantage of starting with a single population that can be reconstructed, for example the four-way cross of inbred lines employed by FALCONER (1953a), is academic because the LCH and LCL are extinct.

Leucocyte counts were made from blood samples obtained from 118 T mice chosen from a total population of 450 animals. From these, 12 females and three males with the highest counts, and 16 females and four males with the lowest counts were selected for polygamous matings. After the original selection (generation 0), the high and low selection lines, destined to become LCH and LCL, were closed except that two females with very high counts were transferred from the low to the high line (generation S₁).

Environmental conditions, though generally favorable, were changed several

times. The influence of these changes on leucocyte count is a matter of conjecture, but for the record the more drastic of the changes are recorded below. After five generations of selection, the colony was transferred from the University of Saskatchewan to the University of Kansas. This change in location was accompanied by a change in diet. At the University of Saskatchewan, a ration formulated by DR. J. M. BELL was used. It contained: 20.1 percent protein, 4.7 percent crude fiber, 7.4 percent crude fat, 1.12 percent calcium, 0.76 percent phosphorus. At the University of Kansas, Purina laboratory chow was fed. It was recorded as containing: 23.0 percent crude protein (minimum), 6.0 percent crude fiber (maximum), 5.0 percent crude fat (minimum), 44.0 percent nitrogen free extract. This diet was supplemented by a mixture of Crisco and two percent brewers' yeast mixed with loose laboratory chow recovered from pellets that had crumbled in the process of handling and storing. This high fat mixture was fed twice a week to breeders and lactating females.

The only major change, other than diet, was the change in procedure for obtaining blood samples to be described below. The blood samples were collected and cells counted by five different operators in addition to the senior author. However, all of the work was done under close supervision and there was no evidence that differences between operators contributed significantly to the error.

Up to the 18th generation, the technique for obtaining blood was as follows: the mouse was placed in a small wire cylinder three inches long by one inch in diameter. One end was closed, the other provided with a tightly fitting cork, notched to accommodate the tail. The extended tail was submerged in water of 45° to 50°C for two or three minutes to dilate the blood vessels. The tail was then dried and one inch to one and a half inches cut off with a sharp razor blade. After the 18th generation, the procedure was to place the mice in a glass jar heated by a 100 watt electric bulb in an overhead gooseneck lamp. When the mice, by their actions, began to show effects of the treatment they were removed and bled by nicking the lateral tail vein. Although this technique might be expected to produce more stress than the former one, it did not seem to introduce any adverse effects. Outside of the main experiment, other procedures were employed, such as bleeding from the heart, but these did not change the order of magnitude of the differences between strains.

The rate of bleeding varied from a steady flow to an oozing; in some cases clotting occurred before a proper sample could be collected. Only good samples were used for cell counts. The first drop of blood was allowed to drop free and then two samples were taken as quickly as possible. No anticoagulant was used and the diluting fluid was one percent acetic acid. Through the first five generations, two types of diluting pipettes were used, Thoma and Trenner. One dilution was made in each pipette from each mouse. The pipettes were alternated so that the first sample taken from one mouse was drawn in a Thoma and the first sample from the next mouse in a Trenner. In the later generations, Thoma pipettes were used exclusively. Immediately after diluting the blood, shaking was done to prevent clumping of the cells and to ensure uniform dispersion. Hand shaking was employed up to generation six after which a Bryan-Garrey pipette

rotor was used. The Spencer Bright-line counting chamber was filled, one side from one pipette, the other side from the second pipette, placed in a moist chamber for ten or more minutes to allow the cells to settle, and counts made on the central and four corner squares of the counting field. The appropriate multiplier in this case, to give the arithmetic count in cells per cubic millimeter, is 40. For analyses, the figures were converted to logarithms, the appropriate scale since new cells arise by mitoses. The average time required for one test was a little less than one-half hour, but MRS. BARBARA MYERS, on a well organized assembly line basis, was able to do better than this. She made all of the counts for generations 7 through 10 and 13. Differential counts, when made, were from slides prepared at the time the blood was collected for total counts. The differential counts were also by standard procedure.

After mice for new matings had been selected, the remainder were inoculated with live *Salmonella typhimurium*. In a preliminary experiment using mice of the first selected generation, dosages of 10,000 and 6,000,000 were used. In the second generation, dosages of 200,000 and 500,000 were used. Mice from generations 3 and 4 and from generations 6 through 18 were all inoculated with the standard dose of 200,000 live organisms. A stock culture known as A-28 was used for the first six tests and a culture known as B-1 for all subsequent tests. The B-1 culture had a lower virulence than A-28, but both cultures could be classed as virulent and formed smooth colonies on nutrient agar plates.

Inoculations were intraperitoneal in all cases and mortality was recorded at least once a day for a period of 21 days.

Surplus mice from generation 5 were exposed to 400r of total body X-irradiation, subsequently sampled for total leucocyte counts according to a prearranged schedule, and finally inoculated with mouse typhoid.

Later experiments were designed to test the resistance of LCH and LCL mice to X-irradiation using a dosage of 650r. The mice were irradiated in a specially constructed polystyrene circular tray divided by radial partitions into ten equal sectors. During exposure, the tray was rotated on a turntable. Dosage rate was determined by placing the Victoreen Roentgen meter on the turntable for one minute of exposure. The dosage rate ranged from 55 to 65r/minute, measured in air. The mice used were from generations 16, 17, 19 and 20.

RESULTS

Response to selection: Selection for high and low total leucocyte counts was practiced for 20 generations. The means and standard errors of 25 generations of the two selected lines are given in Table 1 along with data from T at "generation 0" and corresponding to selected generations 2, 5, 7, 10 and 16. The response to selection is shown in Figure 1 with average number of leucocytes (on a logarithmic scale) of males and females of LCH and LCL plotted against generation number. Selection was effective from the outset in establishing differences between lines with the maximum difference (over 100 percent of the original leucocyte number) attained by generation 13. The intensity of response was

TABLE 1
Total leucocyte counts of LCH, LCL, and T mice

Generation	LCH			T			LCL		
	No. of mice	Mean leucocyte number	Log leucocyte number Mean SE	No. of mice	Mean leucocyte number	Log leucocyte number Mean SE	No. of mice	Mean leucocyte number	Log leucocyte number Mean SE
0				118	9121	3.960			
1	73	9140	3.961 .0157				82	7261	3.861 .0212
2	73	8414	3.925 .0210	29	7925	3.899 .0384	86	6872	3.837 .0166
3	107	8110	3.909 .0160				95	5929	3.773 .0168
4	147	9977	3.999 .0109				143	7499	3.875 .0093
5	97	10642	4.027 .0182	43	7745	3.889 .0225	101	7780	3.891 .0142
6	32	13305	4.124 .0243				74	6039	3.781 .0210
7	96	13032	4.115 .0157	118	8892	3.949 .0146	78	6324	3.801 .0184
8	132	14061	4.148 .0120				42	6592	3.819 .0263
9	89	14521	4.162 .0124				105	6026	3.780 .0158
10	105	14521	4.162 .0163	131	8261	3.917 .0140	154	6295	3.799 .0121
11	99	16368	4.214 .0145				41	4678	3.670 .0203
12	156	14723	4.168 .0111				135	5358	3.729 .0133
13	68	17537	4.244 .0162				42	4000	3.602 .0318
14	85	15702	4.196 .0153				59	4592	3.662 .0242
15	57	14158	4.151 .0237				95	4266	3.630 .0117
16	65	13429	4.128 .0182	51	5105	3.708 .0297	58	4217	3.625 .0239
17	88	11952	4.078 .0146				50	4819	3.683 .0221
18	68	11695	4.068 .0165				56	3556	3.551 .0173
19	38	11852	4.074 .0198				33	3656	3.563 .0171
20	24	11615	4.065 .0401				55	4436	3.647 .0319
21	22	9057	3.957 .0478				51	3891	3.591 .0335
22	25	8395	3.924 .0271						
23	28	9683	3.986 .0186				51	3404	3.532 .0184
24	23	10351	4.015 .0306				36	3681	3.566 .0254
25	16	10423	4.018 .0336				20	3273	3.515 .0263

FIGURE 1.—Response to selection for leucocytes. Mean leucocyte count on a logarithmic scale plotted against generation.

greater, but of shorter duration, in the high line with leucocyte number almost doubled by generation 13. Selection pressure was applied for at least seven more generations but the number of leucocytes actually decreased. Response to selection in the low line appeared to continue until generation 25 when the study was terminated. However, the outbred T mice also followed a downward trend, indicating environmental influences. The magnitude of the selection pressure, i.e., the selection differential, was reasonably uniform in the two lines. Response to selection, plotted against cumulative selection differential, is shown in Figure 2.

The difference between sexes within selected lines was significant in eight of the 49 comparisons: LCH in generations 8 ($P < .01$), 20 ($P < .05$) and 24 ($P < .05$); LCL in generations 8, 12, 16, 19 (all $P < .01$) and 24 ($P < .05$). Lack of consistent direction to the sex differences means that the statistical significance cannot be assigned a biological interpretation.

Frequency distributions were made from logarithmic leucocyte counts and tested for normalcy by the calculation of the third and fourth moments about the mean (g_1 and g_2). Significant deviations from a normal distribution were present in each of the following generations: LCH 3 ($\delta\delta$), 4 ($\delta\delta$), 6 ($\delta\delta$), 8 ($\delta\delta$ and $\delta\delta$), 10 ($\delta\delta$), 13 ($\delta\delta$), 14 ($\delta\delta$), 17 ($\delta\delta$), 18 ($\delta\delta$), 24 ($\delta\delta$) and 25 ($\delta\delta$); LCL 9 ($\delta\delta$), 20 ($\delta\delta$), and 21 ($\delta\delta$). These asymmetrical distributions were consistently leptokurtic and skewed to the right, i.e., more individuals near the mean and more individuals above the mean value than expected. These results are consistent with an hypothesis that uncontrolled environmental in-

FIGURE 2.—Response to selection for leucocytes from Figure 1 plotted against cumulative selection differential. Regression lines fitted by least squares to response from generations 1–13. Generation numbers have been inserted for the points.

fluences generally cause an increase in leucocyte number and that the response to environment is a function of genetic capacity for generating more cells.

Figure 3 shows superimposed representative distributions, with LCH shaded and LCL unshaded. Data from males and females were pooled.

Heritability: There are two principal techniques available for the estimation of heritability, one based on intraclass correlations in the analysis of variance and the other based on regression coefficients in selection experiments. Both methods have been applied to our data.

Sire and dam intraclass correlations were calculated from the mean squares in the hierarchical analysis of variance. For generation 0 (T mice) the 66 mice with leucocyte counts were from five mating cages, each consisting of two females and one male. The estimates of heritability were 22.4 ± 3.44 percent based on the variance among sires, zero percent based on the variance among dams, and 11.2 ± 2.17 percent based on the combined sire and dam variances. The standard errors were calculated by the method described by OSBORNE and PATERSON (1952).

Independent estimates of heritability were calculated as the ratio of selection progress over selection differential. The calculations were confined to the first 13 generations since there was no progress in the high line after that time.

The ratio of the combined (LCH + LCL) selection response and the combined selection differential summed for the 13 generations yielded a heritability estimate of 20.3 percent. This method assumes that the heritabilities are the same in the two lines, but this may not be true (see asymmetry, below).

FIGURE 3.—Frequency distributions of LCH (shaded) and LCL (unshaded) of generations 1, 4, 8, 12, 16, and 20.

Better estimates of “realized heritability” (FALCONER 1953a) can be obtained by use of regression coefficients to estimate average progress per generation. This method minimizes the fluctuations from generation to generation. The differences between the two selected lines in each generation were used to calculate the linear regression coefficients for combined cumulative selection response (CCSR) and combined cumulative selection differential (CCSD). These are shown in Figure 4. The combined cumulative selection differential illustrates the change in leucocyte number that would have occurred if the heritability were 100 percent. The selection differential was weighted by the number of progeny from each of the selected pairs that were measured in the next generation. The calculated regression coefficients were 0.246 ± 0.0070 (CCSD) and 0.0046 (CCSR). The ratio gives an estimate of realized heritability of 18.7 ± 1.85 percent. The standard errors of these estimates were calculated by the method described by

FIGURE 4.—Divergence between LCH and LCL under selection. The upper line is the combined cumulative selection differential and the lower line is the combined cumulative selection response. Regression lines fitted by least squares.

FALCONER and LATYSZEWSKI (1952) and equal the square root of (variance of the regression of response on generation)/(square of the regression coefficient of the selection differential). Confidence limits (95 percent) of this estimate of heritability were 14.7 and 22.7 percent.

A third estimate of 17.4 ± 3.90 percent was obtained from the average of the two regression coefficients shown in Figure 2 where response to selection was plotted against cumulative selection differential. In general, the agreement between estimates obtained from the four independent methods indicates that from a standpoint of selection theory, the results present no unusual features.

The exception is the absence of heritability from variance among T (generation 0) dams in the analysis of variance. No obvious explanation can be advanced to account for the results, since the females were unrelated and age at time of counting cells was controlled. This is opposite to WOLFE's (1961) finding that, for blood-pH in the T line, the heritability estimate was 29.0 percent based on variance between dams and zero percent based on variance between sires.

Test for symmetry: Figure 2 shows the response to selection plotted against the cumulative selection differential. The selection differentials of individual pairs were weighted by the number of offspring measured. Linear regression lines fitted to the first 13 generations gave a measure of the realized heritabilities. The significant difference ($.02 < P < .05$) between these two regression coefficients indicates that the response in the two lines was asymmetrical. The response in the high line was more rapid than that in the low line even though the selection differential in both lines was uniform. Figure 2 also shows a parallelism in the fluctuations of the response in the two lines for the first five generations and after the thirteenth generation. (See also CCSR Figure 4.) It has been shown (FALCONER 1953a,b) that if the scale of measurement is such that the variance is not

independent of the mean, an asymmetrical response will occur. No such association is evident in this study indicating the asymmetry is not a function of the scale of measurement.

Differential counts: Differential counts were made on blood from LCH and LCL mice of generations 7 through 11, 19 through 21, and 23 through 25. Also counts were made on LCH only in generations 18 and 22. In generations 7, 8 and 9, degenerate leucocytes were enumerated separately; there were more degenerates in the LCH line (4.6 percent) than in the LCL line (2.6 percent) (WEIR, COOPER and CLARK 1953).

Lymphocytes, monocytes, neutrophils and eosinophils were enumerated separately. However, monocytes and eosinophils together make up only about two percent of the total, so for analytical purposes the eosinophils have been combined with neutrophils and the monocytes with lymphocytes. The two groups have been designated as "granulocytes" and "agranulocytes," but this classification, based on morphology, makes no assumptions concerning the nature of genetic control over the different cell types.

Since lymphocytes constitute the most numerous cell type in mice, effective selection based on total counts might be expected to apply mostly to this type of cell. Consequently the parallel between the agranulocyte counts (Table 2) and the total counts (Table 1) is not surprising. This also applies to granulocyte number in the LCH line; in the LCL line the number of granulocytes actually increased in absolute numbers and led to an increase in the relative proportion of this type of cell, since the total count went down steadily (Figure 5). Attention

TABLE 2

Differential leucocyte counts of LCH, LCL, T, F₁, and backcross mice

Generation	LCH			LCL		
	No. of mice	Differential counts		No. of mice	Differential counts	
		Agranulocytes	Granulocytes		Agranulocytes	Granulocytes
7	80	9492	1407	50	5296	906
8	123	12927	929	41	5641	742
9	79	13529	1175	91	6012	996
10	66	13131	1400	51	5151	947
11	85	14673	2010	48	3974	811
18	39	9777	2941			
19	43	7746	2603	29	2550	1021
20	29	8401	3116	43	2411	1229
21	13	5413	1926	44	2137	1365
22	25	6246	2502			
23	27	6470	3373	50	2254	1151
24	15	5390	3052	36	2190	1476
25	27	7229	2410	20	1983	1267
Strain or cross	Differential counts					
T	58	6950	1048			
LCH × LCL	42	4580	1005			
F ₁ × LCL	43	3565	2085

FIGURE 5.—Differential leucocyte counts of LCH and LCL mice by generation. Agranulocytes (lymphocytes and monocytes) represented by white bars, granulocytes (neutrophils and eosinophils) by black.

is called to the fact that the absolute number of granulocytes was significantly lower in LCL than in LCH ($P < 0.01$) but not significantly different from T. There seems to be no way to distinguish between the direct effect of selection on granulocyte number and the effect of compensatory regulatory mechanisms.

Corresponding to generation 21 of selection, by which time the lines could be described as inbreds, F_1 mice were counted. Backcross progeny from these $F_1 \times$ LCL were counted at the same time as generation 22 of the selected lines. The data are shown at the bottom of Table 2. The F_1 mice had total counts intermediate between LCH and LCL, being close to the geometric mean of the pure strains, but the granulocyte number was lower than that of the LCL. In the backcross the mean number of leucocytes was not different from the F_1 but the number of granulocytes doubled. Although no definitive conclusions may be drawn from the data, it seems that granulocytes and agranulocytes have different inheritance patterns.

Components of fitness: Our laboratory records include, in addition to the data from various tests, number born, number weaned, weaning weight (30 days), and color.

Litter size at birth with standard deviations are shown in Figure 6. There was

FIGURE 6.—Litter size, means and standard deviations, of LCH and LCL mice.

no perceptible inbreeding depression in either of the lines which would indicate that family selection favored the preservation of a high level of fertility. Also, the LCH and LCL did not differ significantly in litter size. The number of animals available for selection was essentially equal for LCH and LCL and this is reflected by the similarity in selection differentials.

Similarly, the weaning weights for LCH and LCL (Figure 7) paralleled one

FIGURE 7.—Mean weight at weaning of LCH and LCL mice.

another and were not significantly different. Although mature mice were not weighed on a regular schedule, the LCH attained a larger size than LCL. There is evidence from weaning weights, that inbreeding decreased body size. Since the litters were not artificially reduced in number and litter size at birth remained high, these data should be without bias.

Although leucocyte numbers go up with age, our data are based on 30 day counts. Since large differences in leucocyte numbers were not accompanied by body size differences at 30 days, it seems that the genetic control over leucocyte number is independent of influences on growth in general.

A negative correlation between litter size and weaning weight is of common occurrence in polytocous species and the reason is obvious. For the LCH line $r = -.352$ ($P < 0.05$), for the LCL line $r = -.430$ ($P < 0.01$). However, apart from fluctuations from generation to generation, litter size did not show a time trend; therefore the decline in weaning weight must be independent of litter size and may be attributed directly to the effects of inbreeding.

At the conclusion of the experiment, the LCH families were leaden and albino and the LCL were all leaden brown. Albinism disappeared from the low line at generation 6 but persisted in high frequency in the LCH line. In contemporary selection lines, PHH and PHL, the leaden allele also became fixed. These results are consistent with the suggestion of MURRAY (1933) that leaden mice may have an adaptive advantage under certain laboratory conditions. The fixation of b in the LCL line and B in LCH may be due to chance or may be associated with a pleiotropic effect at this locus with the effect of b being to reduce leucocyte number. The same may be said for C although in this case it seems more likely that fixation was a matter of chance.

Resistance to mouse typhoid: Inoculation of mice from generations 1 and 2 with graded dosages of live organisms provided results of a preliminary nature. It was determined that 200,000 organisms was an appropriate dose to establish relative levels of genetic resistance of the strains of mice to infection with *Salmonella typhimurium*. All subsequent tests used this standard dose, but at test 7 the strain of the organism was replaced by one of lower virulence.

In the preliminary tests, there were no survivors from the dosage of 6,000,000 organisms and with a dosage of 10,000 organisms there were: four out of 13 LCH, six out of six LCL, and eight out of 15 T mice. In the test with 500,000 organisms, there were four survivors out of a total of 47 mice in LCH, one out of 55 LCL, and four out of 102 T. Although the ranking of LCH, T, LCL corresponds with expectation on the assumption that leucocyte number *per se* enhances resistance, the differences are not significant and the results of subsequent tests have shown conclusively that the response to selection for total leucocyte count was not accompanied by any change in resistance to mouse typhoid.

The results of the tests, using the standard dose of 200,000 organisms of *S. typhimurium*, are shown in Table 3 and Figure 8. As can be seen from Figure 8, showing results from combining tests within strain of the pathogen, the host genotype had no effect on resistance. Both strains were more susceptible to mouse typhoid than were the outbred T mice. This may be due to the effects of inbreed-

TABLE 3

*Survival of LCH, LCL, and T mice following inoculation with 2×10^5 live organisms of *S. typhimurium**

Test no.	Corresponding selection generation	Strain of pathogen	LCH		LCL		T	
			No. of mice	Percent survival	No. of mice	Percent survival	No. of mice	Percent survival
4	2, 3	A-28	96	39.6	89	33.7	59	42.3
5	3	A-28	25	24.0	23	39.1	32	41.0
6	4	A-28	31	16.1	36	27.8	103	43.7
7	7	B-1	68	19.1	128	17.9	169	72.8
8	6, 7, 8	B-1	62	37.1	15	20.0	42	83.3
11	8, 9	B-1	48	27.1	50	30.0	62	56.4
16	9, 10	B-1	30	36.7	28	50.0	22	81.8
18	11-18	B-1	161	24.2	150	11.3	...	...

FIGURE 8.—Percent survival of LCH and LCL mice inoculated with 200,000 live organisms of *S. typhimurium* of A-28 and B-1 strains.

ing on general vigor, or to the chance fixation of genes for susceptibility to disease. However, this outcome of the investigation is not germane to the problem and the data disprove the hypothesis that leucocyte number *per se* confers resistance.

X-irradiation: The direct effects of X-irradiation on leucocyte number and on residual effects on disease resistance were investigated, using the fifth generation of mice. The initial counts for LCH and LCL before irradiation, but after removal of mice for matings, were not very different with means of 8,870 and 8,020 respectively. The response to irradiation with a dose of 400r, with 74 LCH and 71 LCL did not show any strain difference, but this was no surprise. The fall in leucocyte number (4,400 by the second day after irradiation) persisted for at least a week with a mean count of 4,070 by day 8. Counts were made on days

14 and 15 by which time mean number of leucocytes had risen to 5,390. There were three deaths, all in the LCL strain, and all after the eighth day.

Six weeks after irradiation, the mice were inoculated with the standard dose of live organisms of *S. typhimurium* along with nonirradiated controls. In terms of days to death and percent survivors, the irradiated and control mice were not significantly different (LCH X-ray treated 17 percent, control 16 percent survival; LCL 14 percent and 29 percent survival). The experiment was not repeated but the results show no clear evidence for lowering of resistance to the disease as a result of the earlier X-ray treatment.

Mice from generations 16, 17, 19, and 20 were subjected to X-ray treatment and total leucocyte counts were made by H. D. SWANSON. The dosage employed was 650r and the mice were 30 to 35 days of age. Working with higher dosages of X ray and with inbred mice differing more in initial leucocyte counts than in the earlier experiment, the results should be suitable for valid strain comparisons. Unfortunately the number of mice irradiated was small.

After the acute dose of 650r of X rays, the mice were weighed at intervals up to 30 days, and leucocyte counts made at intervals up to 28 days. Mortality was recorded as it occurred.

The control group consisted of 24 LCH and 19 LCL and these were full sibs of the X-rayed mice. One control mouse died during the experiment. Of 30 X-rayed LCH mice, 24 survived (80 percent) and of 25 X-rayed LCL mice, nine survived (36 percent). The mice with the higher initial leucocyte number, LCH, were significantly more resistant to the lethal effects of X rays than the low count mice, LCL ($\chi^2 = 11.0$, $P < 0.001$). The deaths occurred between days 5 and 15 of the experiment.

Control mice of the LCH strain gained approximately 35 percent and LCL 25–30 percent of their initial weight, whereas all of the irradiated mice lost weight during the 30-day period of observation. Five days after irradiation, the loss was ten percent of the initial weight. The mice that died continued to lose weight until death, and the survivors began to gain weight after the fifth day, exceeding the initial levels by the end of the experiment. The females equaled the controls by 20 days. Since weight-change and mortality data are confounded, weights of LCH and LCL mice are not suitable for comparisons.

Although the LCH mice had twice as many circulating leucocytes as the LCL before treatment, irradiated mice of both strains reached the same low point, 400 cells per cubic millimeter, five to eight days after irradiation (counts lower than 400 cells were found, but only from mice in extremis). Among survivors, recovery of leucocyte numbers usually occurred within a week after this time. In the LCH line, the survivors by day 28 had counts slightly in excess of the initial count, but the controls had also increased (as always occurs, among growing mice). It will be recalled that the mice were highly inbred so that there should be no effects of genetic selection. By 28 days, the LCL had not recovered the initial leucocyte levels. Evidently the high leucocyte count in LCH mice is associated with an ability to produce more cells when needed and this may be responsible

for the higher resistance of LCH than LCL mice to the deleterious effects of total body irradiation.

DISCUSSION

Loss of the LCH and LCL strains of mice cannot be attributed to the effects of inbreeding as fertility and health were satisfactory throughout the investigation, nor can we attribute the loss to management practices during the investigation. After the 25th generation, the lines were carried in small numbers and only enough matings were made to maintain a nucleus. When the time came to expand the colony after moving to new quarters, the available mice were old and failed to produce more than a few litters.

A short review of the results of selection experiments with mice, with references, has been provided by FALCONER (1957). It will be seen from his Table 2, that the heritability of leucocyte count is comparable to that for body weight. The utility of heritability measurements rests on the success with which the breeder may make predictions. Since the predictions based on an analysis of variance of the initial T stock were borne out by the subsequent realized heritability estimates, our selection experiment fulfilled the requirements. Evidently, the precautions taken to reduce environmental fluctuations (control of age at counting would seem to be the most important one) were successful. Since the word "heritability" connotes a direct genotype-phenotype relationship, the fact that it remains stable only under a particular set of environmental conditions is confusing. However, if environmental conditions are optimum, variations of the magnitude of the realized heritability should still persist. An individual from an outbreeding population (e.g. man) may have a genetically determined "high" or "low" leucocyte count without pathological causation just as he may be above or below the mean body weight for his sex and species.

The purpose of the investigation was to produce high and low leucocyte lines of mice to study the contribution of leucocyte number to natural resistance level. Comparable materials from selections based on single criteria would be the PHH and PHL lines of WEIR and CLARK (1955) and toxin resistant lines of HILL, HATSWELL and TOPLEY (1940). Each of the above lines of mice when challenged with live organisms of *S. typhimurium* behaved in a manner similar to the LCH and LCL, i.e., the selection did not enhance resistance to infection. Although mice with a high level of natural resistance to infection have a relatively high leucocyte count (GOWEN and CALHOUN 1943), high blood-pH (WEIR 1949) and high resistance to toxin (GOWEN 1951) there is no evidence that these components act in an additive manner. Our PHH and HILL, HATSWELL and TOPLEY's (1940) toxin resistant lines actually showed greater mortality than the PHL on the one hand and unselected controls on the other.

The characters found in genetically resistant or susceptible strains of mice might be found in association because they act in concert. Unless the characters are genetically or physiologically linked, selection for a single character would not necessarily enhance resistance. This seems to be the case for the examples cited.

There is some evidence for independence of the above named characters (WEIR, COOPER and CLARK 1953). For example, the correlation between leucocyte count and blood-pH was found for 61 T mice to be $r = 0.059$. Mice of LCH and LCL strains were tested for resistance to massive doses of heat-killed organisms. In the first experiment employing 2,000,000,000 B-1 strain killed organisms, survival rates were: LCH, 28 percent; LCL, 29 percent; T (inbred), 34 percent. In the repeat experiment with a dose of 1,500,000,000 killed organisms, survival rates were: LCH, 49 percent; LCL, 54 percent; T (inbred), 61 percent. Nearly all deaths occurred within 48 hours of inoculation. The data together with HILL, HATSWELL and TOPLEY's (1940) findings, indicate that resistance to live organisms and to the effects of toxins are independent components of resistance. Our LCH and PHH mice did not differ significantly from LCL and PHL mice in resistance to live or heat-killed organisms; their genetically toxin resistant mice were on the average uniformly more resistant to live organisms (64.2 percent survival) than were the controls (14.0 percent survival).

Although selection for high and low leucocyte count was not accompanied by changes in natural resistance to a first infection, there remained the possibility that LCH and LCL mice might differ in ability to acquire immunity. To test this hypothesis, an experiment using 209 mice, with approximately equal numbers per strain, was performed. The mice were immunized by sublethal doses of vaccine and subsequently challenged with 200,000,000 live organisms. Survival rates were: LCH, 76 percent; LCL, 79 percent; T (outbred), 77 percent; T (inbred), 84 percent. Agglutination tests failed to show consistent strain differences in antibody titer. These data do not support the hypothesis.

The results from exposure to 650r of X rays present a different picture and in retrospect this should not be a surprise. Mice have been subjected to a long history of exposure to infection; an acute dose of X rays is an artificial insult. On the one hand, mobilization of cellular elements along with complex reactions at the molecular level serves to combat specific diseases, on the other hand, the cells themselves are targets for destruction. The significantly higher survival rate of LCH than LCL, following acute X-irradiation, (both fell to the same level of 400 cells per cubic millimeter) indicates that LCH mice not only have more circulating leucocytes than LCL, but have more "resistant" stem cells.

High leucocyte count strains of mice may, in general, be more resistant to the effects of irradiation than low count mice, but they are not necessarily more resistant to mouse typhoid. The three strains, S, Z, and Ba, of GOWEN and CALHOUN (1943) subjected to X rays (STADLER and GOWEN 1957) showed similar ranking in leucocyte count, resistance to irradiation and resistance to mouse typhoid. However, the strains also showed a relation between normal leucocyte count and resistance to mouse typhoid in the absence of irradiation. If we assume that these strains possess qualitative differences in leucocytes not present in LCH and LCL, the findings are compatible. One of STADLER and GOWEN's (1957) strains, known as Q, was comparable to the *Salmonella* susceptible Ba mice in reaction to mouse typhoid, but resembled the resistant S strain in level of resistance to X rays.

Reasons for the higher survival of LCH than LCL to the effects of an acute dose of X rays are unknown. Since we are not now in a position to carry out further experiments, we can only speculate. Since differential counts were not made, we can say little about the role of granulocytes, but it is generally recognized that the drop in leucocyte count of mice is a lymphopenia. The importance of the spleen is also well known. However, since animals dying from acute radiation sickness show effects that are referable to the gastrointestinal tract (particularly the duodenum and jejunum), it seems reasonable to focus attention on this area. Lymphocytes are eliminated by passage through the intestinal wall. AMBRUS and AMBRUS (1959) have postulated that disintegrating cells may release antibodies that aid in controlling bacterial flora. Presence of more lymphocytes in the intestinal lumen to begin with, augmented by addition of cells killed by irradiation, may afford a transient protection of LCH mice at a critical stage.

SUMMARY

1. The mean of total leucocyte counts from 118 outbred mice (T strain) was 9121 cells per cubic millimeter (log count $3.960 \pm .0128$).

2. Selections were based on family and individual merit in a closed system and matings were brother by sister.

3. By generation 13, the maximum difference between strains was achieved with arithmetic and log counts as follows: LCH 17,500 ($4.244 \pm .0162$); LCL 4,000 ($3.602 \pm .0318$).

4. Although the selection differential was uniform in the two lines, the intensity of response was greater in LCH. Response in the LCL was more gradual, but continued until the end of the experiment. However, the outbred T mice also followed a downward trend, indicating environmental influences.

5. Heritability estimates by four methods were: 22.4, 20.3, 18.7, and 17.4 percent.

6. The major portion of the difference in leucocyte count between LCH and LCL is accounted for by lymphocytes. LCH mice had more cells of all categories. In LCL mice the number of granulocytes increased under downward selection pressure for total count.

7. The selected lines did not differ significantly in numbers of progeny or weaning weight. At the end of the experiment, LCH families were albino and leaden, LCL leaden brown.

8. There were no significant differences between strains in resistance to a standard dose of live organisms of *S. typhimurium*, the toxic effects of massive doses of heat-killed organisms, or to reinoculation with live organisms after immunization.

9. After an acute dose of 650r of X rays, survivals were: LCH 24/30 (80 percent); LCL 9/25 (36 percent).

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