The Nature of Genetic Resistance to Infection in Mice[1]

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Selection experiments have been devised to show the relation of total leucocyte count and blood pH in mice to resistance to mouse typhoid. Results of Hill *et al.* (*1*), with respect to resistance of mice to endotoxins, have also been considered.

A number of inbred strains of mice exhibit characteristic and relatively stable responses to infection with *Salmonella typhimurium*. The Iowa State College genetics laboratory, for example, maintains 12 such strains, 6 of which have been utilized over a period of years to investigate the physical basis of natural resistance (*2*). A rather definite picture has emerged. In general, resistant strains differ from susceptible in possessing relatively higher leucocyte counts (*3*), higher blood pH (*4*), and greater resistance to the toxic effects of massive doses of heat-killed organisms (*5*).

Since the mouse strains employed by J. W. Gowen and others were first differentiated on a basis of resistance or susceptibility to mouse typhoid, they are unsuitable for critical evaluation of independent contributions of single mechanisms to the over-all resistance complex. To attack this aspect of the problem, selection experiments were inaugurated at the University of Saskatchewan in 1949 to produce a high leucocyte line (LCH) and a low leucocyte line (LCL) and to produce high and low blood pH lines (pHH and pHL). MacArthur's random-bred mice (*6*) were chosen as the initial breeding stock, since they were originally derived from crossing 6 distinct laboratory strains, none of which had been previously employed in disease-resistance studies. They have been designated the T strain and are being maintained as an outbred control line. In 1950 the entire mouse colony was transferred to the University of Kansas, at which time some family lines were lost. Selection has been coupled with intensive inbreeding, mostly by full-sib matings.

Divergence of high and low leucocyte lines under the influence of selection with inbreeding is illustrated in Table 1. Since age is a major source of nongenetic variation, counts were made when mice were 30 days old or within 2 or 3 days of that age. Metrical bias, resulting from peculiarities associated with the technique of bleeding from the tail, is judged to be of no importance, since the order of magnitude of differences between strains was not altered when blood was collected by cardiac puncture or when procedure was varied in other ways. Data presented in this paper were obtained by a standard technique involving tail blood, so that our actual figures are not necessarily comparable with those of other workers. We believe that systematic errors are likely to persist in long-term experiments despite all efforts at randomization, but by using controls and, in the case of resistance tests, repeating experiments, bias can be minimized.

It was not feasible to count T mice each time the selected mice were counted, but counts were made at the same time as the 2nd, 5th, and 10th selected generations. It will be noted that the mean cell count in the outbred T line has remained reasonably stable (Table 1). It is therefore likely that environmental factors contribute relatively little to the variance attributable to differences between generations.

TABLE 1

SELECTION FOR HIGH AND LOW LEUCOCYTE COUNTS IN MICE
(Total Leucocyte Counts/mm³ from Tail Blood)

Generation	LCH strain		LCL strain		Difference	T strain	
	No. of mice	Mean cell count SE	No. of mice	Mean cell count SE	LCH—LCL	No. of mice	Mean cell count SE
0						118	9,599 ± 288
1	73	9,564 ± 340	82	7,956 ± 366	1,608		
2	73	9,169 ± 414	86	7,299 ± 273	1,870	30	9,503 ± 787
3	107	8,685 ± 309	97	6,321 ± 228	2,364		
4	147	10,470 ± 274	143	7,769 ± 172	2,701		
5	97	11,520 ± 456	103	8,074 ± 257	3,446	43	8,212 ± 460
6	32	13,902 ± 717	74	6,539 ± 309	7,363		
7	96	13,832 ± 482	78	6,767 ± 293	7,065	118	9,482 ± 308
8	132	14,710 ± 379	43	7,103 ± 427	7,607		
9	89	15,021 ± 407	101	5,729 ± 273	9,292		
10	105	15,525 ± 524	154	6,674 ± 194	8,851	131	8,798 ± 250

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Differential counts made on blood from mice of generations 7, 8, and 9 showed consistent differences between LCH and LCL lines, with LCH mice exhibiting higher lymphocyte and lower neutrophil propor-

TABLE 2

DIFFERENTIAL LEUCOCYTE COUNTS FOR 3 GENERATIONS OF LCH AND LCL MICE

Generation	Mouse strain	No. of mice	Lymphocytes	Monocytes	Neutrophils	Eosinophils
7	LCH	68	84.5	1.1	12.8	1.5
	LCL	26	82.8	3.3	13.0	.8
8	LCH	129	90.9	1.2	7.2	.6
	LCL	43	86.7	1.5	10.7	.8
9	LCH	80	90.8	.5	8.3	.4
	LCL	84	84.8	.7	14.0	.5
7, 8, and 9	LCH	277	89.3	1.0	8.9	.8
	LCL	153	85.0	1.4	12.9	.7

tions than LCL mice. Degenerate leucocytes, which were enumerated separately, were more numerous in the high line (4.6%) than in the low (2.6%). Results are shown in Table 2. Despite the differences in differential counts, the LCH strain exceeded the LCL in all categories on a basis of absolute numbers of cells, indicating that selection was effective for all types of cells.

We were confident at the outset that high line animals would be relatively resistant and low line ones relatively susceptible to mouse typhoid. In fact, early tests, involving small numbers, did suggest such an interpretation, which was reported (*7*). It is now evident that the predicted relationships do not hold. High line mice do differ from lows in resistance to mouse typhoid but in an inverse manner. The data concerning inoculation tests, employing 200,000 live organisms of *S. typhimurium,* are summarized in Table 3. The first three tests were done at the University of Saskatchewan, the last three at the University of Kansas; the main difference involved a change to a strain of pathogen of lower virulence. Experimental control over dosage and other nongenetic variables was effective, as evidenced by repeatability in the T strain, particularly in tests employing A-28 pathogen. It might be argued that the difference in resistance between the outbred T and the inbred LCH and LCL lines is a function of mating system and not related to leucocyte count per se. This view finds support in results of resistance tests employing an inbred line also derived from T, but in which no selection had been practiced (Table 3). Regardless of our interpretation of the data, it is evident that high leucocyte count does not, by itself, confer an advantage to the host from a standpoint of resistance to mouse typhoid. Genetically high leucocyte count mice were, in fact, at a disadvantage. It is therefore suggested that in the inbred lines employed by Gowen and Calhoun (*3*) genes have been fixed which affect quality of leucocytes, whereas in our selection experiment leucocyte number alone has been altered. Genetic differences in ability of leucocytes (*8*) and macrophages (*9*) to digest phagocytosed bacteria, as well as other qualitative properties of leucocytes, are known (*10*).

TABLE 3

SURVIVAL OF LCH, LCL, AND T MICE FOLLOWING INTRAPERITONEAL INOCULATION WITH 200,000 LIVE ORGANISMS OF *S. typhimurium*

		LCH		LCL		T		Inbred T	
Test No.	Strain of pathogen	No. of mice	% survival	No. of mice	% survival	No. of mice	% survival	No. of mice	% survival
4	A-28	100	40.0	92	32.6	59	42.3		
5	A-28	21	19.0	23	39.0	32	41.0		
6	A-28	31	16.1	36	27.8	103	43.7		
7	B- 1	64	18.8	130	24.6	169	72.8	35	51.4
8	B- 1	61	36.1	15	20.0	42	83.3	7	28.6
11	B- 1	48	27.1	51	33.3	62	56.4	45	42.2
		325		347		467		87	

TABLE 4

SURVIVAL OF pHH, pHL, AND T MICE FOLLOWING INTRAPERITONEAL INOCULATION WITH 200,000 LIVE ORGANISMS OF A-28 *S. typhimurium*

Test No.	pHH		pHL		T	
	No. mice	% survival	No. mice	% survival	No. mice	% survival
4	154	29.8	159	36.5	59	42.3
5	39	28.2	42	42.8	32	41.0

Although extenuating circumstances might be invoked to account for the results with respect to leucocytes, a different set of arguments would be necessary to explain an equally unsuspected relationship between blood pH and resistance. Again, starting with outbred T mice, selection and inbreeding were practiced to produce high and low lines, but in this case blood pH was the criterion of selection. Employing the technique described by Weir (*4*), Clark (*11*) was successful in one generation in separating out a high line (pH 7.47 ± .005) and a low line (pH 7.42 ± .005) from the T strain (pH 7.45 ± .004). Two additional generations of selection resulted in no appreciable change in the means. Also, inbreeding was no more effective than assortative mating in fixing genes affecting blood pH, indicating that the genetics of blood pH is not complex. Results of resistance tests employing a 200,000 dose of A-28 strain *S. typhimurium* are shown in Table 4. As in the case of leucocyte count, the high line proved to be more susceptible to mouse typhoid than did the low line, a result not to be anticipated on a priori grounds, in view of the positive correlation between blood pH and resistance reported to exist in the Iowa State College inbred mouse strains (*4*). The possibility of a subtle interaction between blood pH and the effectiveness of leucocytes cannot be precluded, but no correlation between leucocyte count and blood pH was found for 61 T mice tested for both

($r = .059$), indicating independent genetic determination of the two characters.

Finally, an independent example of the nonadditive nature of *resistance components* comes to us from the results of an investigation in which the aims were quite different from our own. Hill *et al.* (*1*), disturbed by the possibility that residual congenital passive immunity might influence inheritance studies, undertook to employ a partially purified toxic fraction isolated from *S. typhimurium*. As in our experiments, marked response to selection occurred. The survival rates among toxin-injected mice (combining 9th and 10th generations) were 64.2% for the selected group compared to 14.0% for the control group. When live organisms were employed on similar mice, either *per os* or intraperitoneally, the results were "very surprising." The genetically toxin-resistant mice were on the average uniformly more susceptible to the living organism than were the controls. Unfortunately the experiment was terminated by the outbreak of the war, but the parallel with our independent results concerning leucocytes and blood pH is striking.

To test the suggestive hypothesis that a physiological relationship between leucocyte count and toxin resistance might exist, mice of LCH, LCL, and T strains have been subjected to massive intraperitoneal injections of vaccine. The results have been negative in terms of the hypothesis. In the first experiment (Test 9) employing a dose of 2,000,000,000 killed organisms, survival rates were: LCH, 28%; LCL, 29%; T (inbred), 34%. In Test 10, with a dose of 1,500,000,000, survival rates were: LCH, 49%; LCL, 54%; T (inbred), 61%. Nearly all deaths occurred within 48 hr of inoculation. Although the mouse strains reacted in the same manner to toxins as to live organisms, there still existed a possibility that there might be strain differences in ability to acquire immunity. Accordingly, mice of the 3 strains were immunized by sublethal doses of vaccine and subsequently challenged with 200,000,000 live organisms. Survival rates were: LCH, 76%; LCL, 79%; T (outbred), 77%; T (inbred), 84%. This experiment has not been repeated, but the test involved 209 mice with approximately equal numbers per strain. Agglutination tests failed to show consistent strain differences in antibody titer.

Some degree of genetic resistance to a naturally occurring mouse disease, as a result of past natural selection, is to be expected in any population of mice. Moreover, components of resistance are likely to be balanced in some manner. Artificial selection, based on ability to survive infection, will accomplish essentially the same result as a naturally occurring epidemic and should also lead to balanced gene combinations. This will not be true in the case of selection experiments directed toward altering a single character unless this character is directly adaptive. If leucocyte number, for example, affected resistance in general, then selection for high count should lead to enhanced resistance. Our investigation supports the view that resistance to mouse typhoid is dependent on complex interactions of characters which, taken individually, do not confer resistance to the host.

Nonspecific mechanisms of resistance to bacterial diseases are likely to be common to mice in general, so that isolation of genetic components will be possible only in those rare cases which involve a mutation in an essential gene. Lack of complement in guinea pigs, resulting from a defective recessive gene, affords an example (*12*). Complement-deficient animals were found so susceptible to a variety of diseases that the line could not be maintained even under laboratory conditions.

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A High-Temperature Strain of *Chlorella*

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Various strains of the green alga *Chlorella* have been used extensively for study of photosynthesis and other physiological processes. Such work has been restricted almost exclusively to temperatures at or below 25° C. The choice of lower temperatures, probably made on the basis of qualitative experience, is consistent with the ecological observation that "many algae do not survive a rise in temperature and thrive only in cold waters" (*1*).

Recent investigations on the mass culture of algae have demonstrated the difficulties of maintaining temperatures as low as 25° C in dense suspensions under sunlight illumination (e.g., [*2*]). An alga with a higher temperature optimum would be of obvious usefulness for both practical application to mass culture and experimental application to physiological studies. We are now able to report the isolation of a number of strains of Chlorella with higher temperature optima and the salient characteristics of one of the strains.

Samples from warm local surface waters were used to supply inocula for test tube cultures grown in a Knops solution at pH 6.8; the medium was provided with microelements and 0.5 g/liter of a chelating