THE INHERITANCE OF RESISTANCE TO *SALMONELLA AERTRYCKE* IN VARIOUS STRAINS OF MICE[1]

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INTRODUCTION

Disease resistance in animals is a field in which there has been relatively little investigation from the genetic point of view. With the development of more precise methods for laboratory control of diseases and of heredity, more critical experiments from the genetic standpoint are demanded of the animal pathologist.

The experiments reported in this paper were planned to investigate the factors involved in reaction differences among various laboratory strains of mice when subjected to a controlled infection with a specific bacterial disease. Selection for resistance was the major object of the investigation using the survival of progeny as a criterion for the breeding in successive generations. Although this study was concerned exclusively with reactions inherent in mice, precedent for the general plan of investigation was drawn directly from experiments on rats conducted in the Genetics Laboratory by IRWIN (1928, 1929) and on poultry by LAMBERT and KNOX (1928).

The increased resistance among rats to the Danysz bacillus observed by IRWIN over three generations of selection was attributed to complex genetic factors some of which apparently were partially dominant in inheritance. LAMBERT and KNOX, using the reaction in chicks as a basis for selection, also suggested that multiple genetic factors, in part, determine resistance to fowl typhoid. ROBERTS and CARD (1926), likewise working with chicks, showed that resistance to bacillary while diarrhea was definitely influenced by heredity. Preceding this, FRATEUR (1924) had proposed a single factor for determining resistance to avian diphtheria. Among highly inbred lines of guinea pigs, WRIGHT and LEWIS (1921) noted appreciable differences in reaction to tuberculosis.

In reports dealing with bacterial infections of mice, various authors have observed individual as well as strain and stock differences in resistance. TYZZER (1917) and HAGEDOORN-LABRAND and HAGEDOORN (1920) observed that during laboratory epidemics the Japanese waltzing mouse stocks were much less resistant to infections than were other mouse stocks. PRITCHETT (1926) reported distinct differences of reaction between inbred

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strains of mice when subjected to controlled doses of paratyphoid-enteritidis and Type II mouse typhoid (*Bacillus pestis caviae*).

Among the investigations dealing with infections of mice, WEBSTER (1924, 1925) has presented the most clear-cut evidence of heredity as a factor in resistance. He has shown that by selective breeding resistance could be increased or diminished when using paratyphoid-enteritidis as the infective agent.

MATERIALS AND METHODS

The mouse (*Mus musculus*) was selected as a subject for this study because of its wide use in pathological work, its adaptability to laboratory conditions, and its extensive use in hereditary investigations. In 1928, when this study was initiated, the strains available for tests were the Bagg albino, Strong dark-brown, Little dilute-brown, and a commercial strain designated as (Sch) Schwing albino. Later three other lines were acquired, Short-eared, pink-eyed, dilute-brown; English silver; and a white-faced, or piebald strain.

The foundation stocks for the strains came from various sources as follows: Doctor E. C. MACDOWELL of the CARNEGIE INSTITUTION OF WASHINGTON supplied the Bagg albino (Ba) strain in 1924. This strain had been developed by Doctor H. J. BAGG of MEMORIAL HOSPITAL, New York in 1916, and had been inbred by MACDOWELL since 1922. Other stocks provided by MACDOWELL were the Little dilute-brown (Li) strain in 1926; a short-eared, pink-eyed, dilute-brown (Pbr), and an English silver (Sil) strain early in 1929. The Li strain was developed by Doctor C. C. LITTLE while at the UNIVERSITY OF MAINE; the Pbr strain was isolated by Doctor CLARA J. LYNCH of the ROCKEFELLER INSTITUTE; and the Sil strain was from stock imported from England in 1927 by Doctor L. C. DUNN of COLUMBIA UNIVERSITY. The Strong dark-brown (Str) strain originally came from Doctor L. C. STRONG of the BUSSEY INSTITUTION in 1926. The Schwing albino (Sch) strain came from Mr. ED SCHWING, a dealer in Harrisburg, Pennsylvania, in 1927. The white-faced (Wf) strain came from BUSSEY INSTITUTION stock in 1930 through courtesy of Doctor M. R. IRWIN, now of the UNIVERSITY OF WISCONSIN.

All of the strains except the Sch and Sil have been continued by brother-sister or parent-offspring mating. The Sch strain has been carried as a pen-inbred line. Progeny from all of the seven strains have been used in tests as described in the body of this report.

The disease organism used in this study is *Salmonella aertrycke*, a species placed by WELDIN (1927) and by BERGEY (1930, pp. 339–350) in the genus Salmonella of the colon-typhoid group of bacteria. It is well known as

a specific typhoid producer in mice and as a producer of typhoid-like diseases in other laboratory animals. Furthermore, it is extensively associated with food poisoning in man (JORDAN 1931, pp. 144–147, and SAVAGE 1925). A culture was supplied to us in 1926 through the courtesy of Doctor W. W. C. TOPLEY, PUBLIC HEALTH LABORATORY, Manchester, England. TOPLEY and his associates have carried on extensive epidemiological and virulence studies with this organism using the mouse as the host (TOPLEY 1925, LOCKHART 1926, WILSON 1930). A stock of the organism used in the Genetics Laboratory is carried in the American Type Culture Collection, JOHN MCCORMICK INSTITUTE, listed under material supplied by M. R. IRWIN, 1926, as *S. aertrycke*, No. 854—"2188 (Type)."

In our laboratory the organism has been carried by monthly transfers on veal infusion agar slants. For each inoculation an 18 hour culture was suspended in 5 cc physiological salt solution and transferred to a sterile stock bottle. A sample was then drawn from the stock bottle and heated for 20 minutes at 55–60°C to render the organism non-motile. The number of organisms was then determined by use of a Petroff-Hausser bacteria counter, and the original suspension diluted so that the required number of bacteria were contained in 0.25 cc of physiological salt solution.

The method of inoculation in all cases was by intraperitoneal injection, the inoculations being made immediately after the dilution was complete. This procedure was considered as a direct and controllable means of uniform infection with the disease.

At approximately 60 days of age the animals were weighed and taken into the isolation laboratory. After inoculation the animals were checked each morning and mortalities recorded over a period of 21 days.

STANDARDIZATION OF DOSAGE

At the outset of this study it was not known how large a dose could be given to an individual mouse of any given strain and still allow for survival and reproduction of a few individuals within the tested group. Accordingly, at consecutive times when animals were available, doses of various fixed numbers were administered.

A suggestion for the usable range of dosage was drawn from LOCKHART'S (1926) summary of mortalities under various doses of the organism. Following intraperitoneal inoculation with graded doses LOCKHART'S experiments show:

Dose	*Number of mice at risk*	*Percentage mortality at fourteenth day*
10^7	400	90.75
10^5	400	70.00
10^3	400	59.75
10	400	30.75

The results of the various doses used in our laboratory on the Schwing strain are summarized in table 1. Here it is shown that a dose of 2×10^5 organisms or over allows for little survival, and it is of interest to note that none of these survivors produced offspring. Doses of 5×10^4 organisms permitted some survival followed by ability to reproduce. The lowest dose administered, 10^4 organisms, allowed distinctly more survival. However, the survivors of this low dose were not used in these experiments because it was believed that animals surviving the next higher dose would possess more hereditary factors for natural resistance.

TABLE 1

Percentage mortality in Sch mice following inoculation with different numbers of S. aertrycke.

NUMBER ORGANISMS IN DOSE	NUMBER INOCULATED	NUMBER DEAD	PERCENTAGE MORTALITY
1×10^7	64	64	100.0
2×10^6	111	109	98.2
2×10^5	228	219	96.0
5×10^4	538	443	82.3
1×10^4	102	71	69.6

The large number of animals listed as tested under the 5×10^4 dose is an accumulation of groups used throughout the experiment as checks for testing the consistency of the virulence of the organism. The survivors from the earlier tests with this standard dose were the progenitors of succeeding generations of our resistant strain.

The rate of mortality occurring under each of the various doses is presented in figure 1. The death rate in each group is expressed in daily percentage mortalities over the test period of 21 days.

The mortality curve of the highest dose can be interpreted as the result of an acute form of infection. The curves of the 2×10^6 and 2×10^5 doses show few differences. The small number of animals in these two groups living over 14 days can probably be ascribed to their sustaining a sub-acute infection. In the reaction given by the lowest dose, 10^4 organisms, the sharpest rise is before the fourteenth day, but the subsequent flattening of the curve is less marked than under the standard dose.

Another striking feature shown by the curves is the similarity of the incubation period in the doses of 2×10^6, 2×10^5, and 5×10^4 organisms. The first mortality occurs on the third day in all three cases. With the dose of 10^7 organisms fatalities are evident on the first day, while with the lowest dose virtually no mortalities occur until the seventh day.

Another feature of the standard dose may be noted in the proportionate differences between the mortalities of the various groups. These statistics

have been calculated according to FISHER's (1930, p. 84) tests for independence and are given in table 2. The difference between the standard dose (5×10^4) and the one four times as large is well beyond that expected from random variation. The same can be said of the standard dose in relation to the one which is five times smaller.

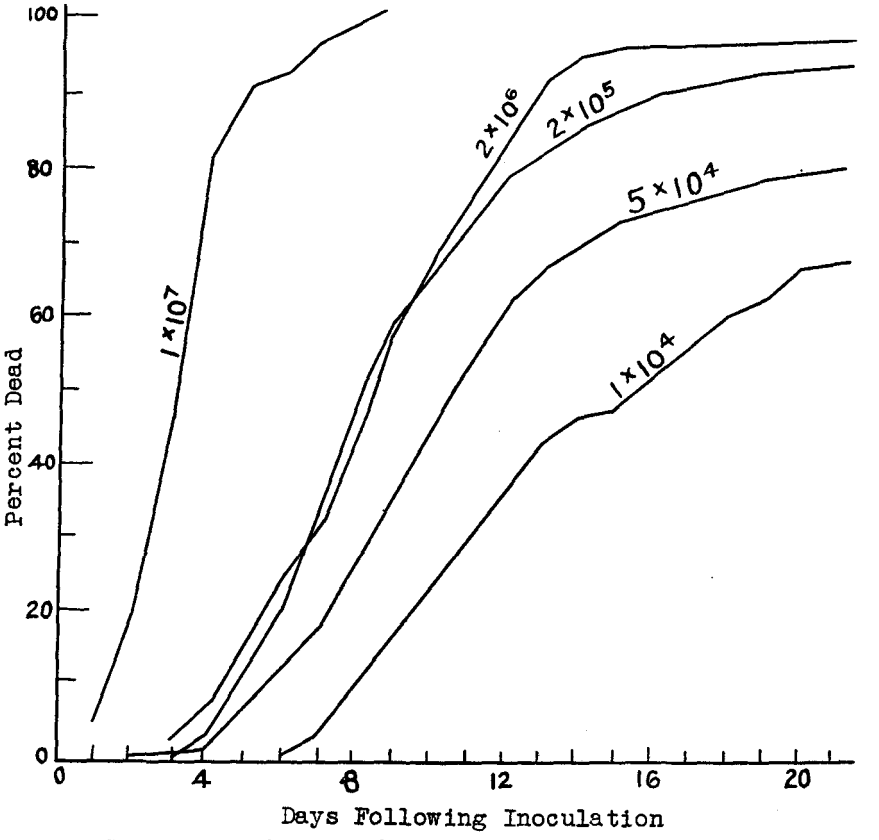

FIGURE 1.—Death rates in Sch mice over a period of 21 days following various doses of *S. aertrycke.*

TABLE 2

Comparison of the differences in percentage mortality resulting from various doses of S. aertrycke.

DOSES COMPARED	DIFFERENCES IN PERCENTAGE MORTALITY	X^2	P
10^7 and 2×10^6	1.8	2.024	0.16
2×10^6 and 2×10^5	2.2	3.424	0.07
2×10^5 and 5×10^4	13.7	26.738	$\pm.01$
5×10^4 and 10^4	12.5	8.946	$<.01$

Simultaneously with the preliminary tests on the Schwing mice, animals from the Bagg albino strain were inoculated with the three lower doses. Likewise Strong dark-brown entered the tests under the 2×10^5 and 5×10^4 doses. The results from these tests are summarized in table 3, and the mortality rates are presented in figure 2.

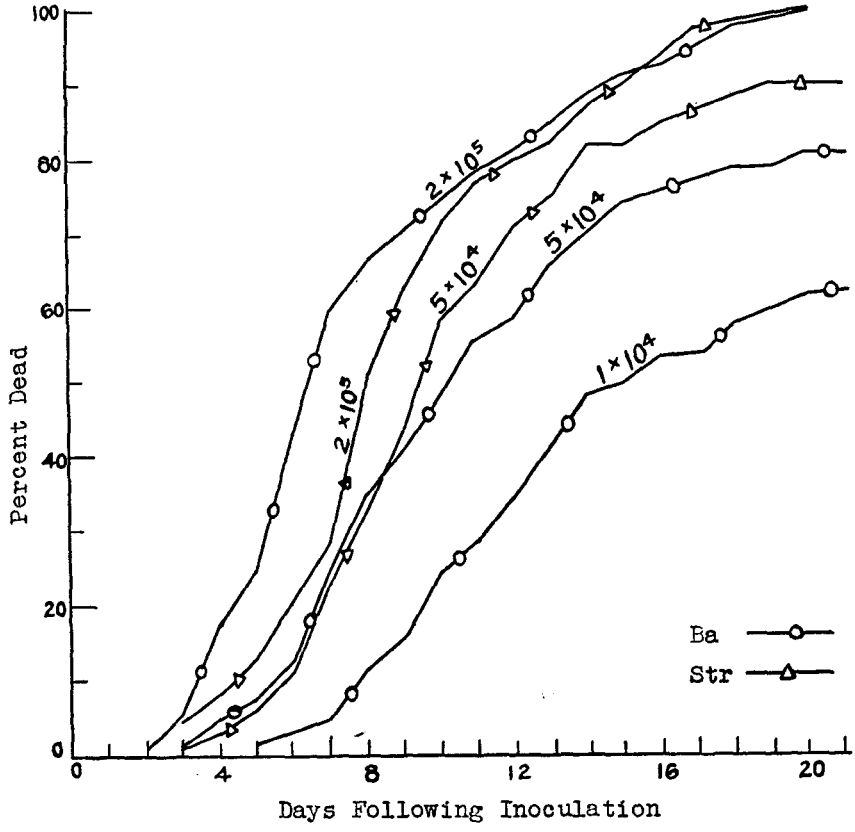

FIGURE 2.—Mortality rate curves in Bagg albino and Strong dark-brown stocks following inoculation with varying doses of *S. aertrycke*.

It should be noted that although the Ba strain is more highly inbred than the Sch strain the mortality in each strain under similar dosage is almost identical (see tables 1 and 3). The reaction of the Str strain is like that of the Ba in the 2×10^5 dose, but with the standard dose the mortality is appreciably higher.

The reactions of these three strains of mice under the various doses were taken as an index of the utility of the 5×10^4 dose as a standard.

TABLE 3

Percentage mortality in Ba and Str strains following various doses of S. aertrycke.

DOSE	STRAIN	NUMBER INOCULATED	NUMBER DEAD	PERCENTAGE MORTALITY
2×10^5	Ba	90	90	100.0
2×10^5	Str	78	78	100.0
5×10^4*	Ba	97	78	80.4
5×10^4*	Str	54	49	90.7
1×10^4	Ba	56	35	62.5

* Standard dose.

REACTIONS UNDER THE STANDARD DOSE

A statistical method for use when the observations are expressed in percent is illustrated with the data in table 4 and the following formulae. The method, as developed from various statistical considerations by Professsors G. W. SNEDECOR and A. E. BRANDT of the Mathematics Department, IOWA STATE COLLEGE, tests a series of percentage probabilities for homogeneity. In cases of non-homogeneous material, the method may reveal the character of the variation.

TABLE 4

The percentage dead in 13 samples of Sch mice inoculated with the standard dose, 5×10^4 organisms.

NUMBER INOCULATED (I)	NUMBER DEAD (D)	PERCENTAGE MORTALITY (p)
40	34	85.00
39	27	69.23
68	59	86.76
62	51	82.23
76	59	77.63
32	30	93.75
20	16	80.00
66	61	92.42
40	31	77.50
36	27	75.00
27	20	74.07
12	11	91.66
20	17	85.00
538	443	82.34

The sample of 538 Sch mice was tested in sub-samples at thirteen different times. The number inoculated at each time is listed under I, the number dead under D, and the percentage dead under p.

Other symbols used in the formulae are as follows:

$\bar{p}$ = the weighted mean mortality percentage.
$\bar{q} = 100 - \bar{p}$.
N = the number of sub-samples.
n = the average number in the sub-samples.
σ = the weighted standard deviation in percent.
σ_B = the Bernoulli standard deviation.
L = the ratio of $\frac{\sigma}{\sigma B}$ (Lexian ratio).

Then

$$\sigma^2 = \frac{100 \Sigma Dp}{\Sigma I} - \bar{p}^2 = \frac{100 \times 36745.41}{538} - (92.34)^2 = 50.13$$

$$\sigma_B{}^2 = \frac{\bar{p}\,\bar{q}}{n} = \frac{82.34 \times 17.66}{41.38} = 35.14$$

$$L^2 = \frac{50.13}{35.14} = 1.4265$$

$$L = 1.19$$

When $L = 1$ a series is interpreted as having a binomial distribution of percentages, the probability of death being constant throughout the sample. With $L > 1$ a series is said to be supernormal, and is interpreted as due to variation of probability of death from one sub-sample to another. When $L < 1$ the series is subnormal, the probability varying from individual to individual in each sub-sample, the same series of probabilities being repeated from sub-sample to sub-sample.

The X^2 test for homogeneity furnishes a probability that the variation in percentage dying is due to random sampling. To apply this to the data above:

$$X^2 = NL^2$$
$$= 13 \times 1.4265 = 18.544$$

Then from FISHER'S (1930, p. 96) X^2 tables, the above X^2 value is found under $n = 12$, that is, $N - 1$, and the corresponding $P = 0.10$.

This value of P means that a X^2 of 18.544 or larger would be expected from random variation 10 times per hundred in similarly drawn samples. In other words, the variation in mortality percent among the sub-samples in table 4 is well within that attributable to random sampling.

It may be noted that the grouping of the Sch mice into thirteen samples results from routine testing and is entirely arbitrary. A more natural basis for the sub-sample is the litter. Individual records on 25 litters of Sch mice

are available. A comparison of these litters with the whole population and with the three test groups into which they fall is as follows:

	Total number	*Number dead*	*Percentage mortality*	*L*	X^2	*P*
13 test samples	538	443	82.3	1.19	18.54	0.10
25 litters	107	87	81.3	0.97	23.91	0.47
The 25 litters in 3 test groups	107	87	81.3	0.69	1.44	0.49

Here the different classifications show no tendency to disturb the binomial distribution. However, in the Ba mice we have:

	Total tested	*Number dead*	*Percentage mortality*	*L*	X^2	*P*
30 litters	97	78	80.4	1.20	43.80	0.04
In 4 test samples	97	78	80.4	1.03	4.26	0.23

The smallness of the probability and the supernormal Lexian ratio under the litter grouping suggest that there may be significant differences in probability of death from litter to litter in this strain.

A summary of the reactions of the various strains tested with the standard dose is given in table 5.

TABLE 5

Percentage mortality and test for type of dispersion within each of the strains under a standard dose of S. aertrycke (5×10^4 organisms).

STRAIN	NUMBER INOCULATED	NUMBER DEAD	PERCENTAGE DEAD	NUMBER OF LITTERS	L	X^2	P
Sch	534	443	82.3	13*	1.19	18.54	0.10
Ba	97	78	80.4	30	1.20	43.80	0.04
Str	54	49	90.7	19	0.98	18.67	0.48
Li	56	54	96.4	14	0.97	13.23	0.36
Pbr	86	73	84.7	19	1.61	43.62	0.01
Sil	108	108	100.0	..	..	..	..
Wf	71	71	100.0	..	..	..	..

* Number of test samples.

Three of the strains, the Sch, Ba, and Pbr, show approximately the same total mortality. The P of slightly less than 0.01 for the Pbr strain suggests some variation from litter to litter. The Sil and Wf strains show total susceptibility, while the other two strains show a reaction intermediate between the higher and lower figures. In so far as is known, the Wf, Pbr, Li, Str, and Ba strains are more highly inbred than the Sch and Sil strains, but the observed mortality would seem to indicate no difference between the strains on the basis of the degree of inbreeding.

The percentage mortality rates of the seven groups are given in figure 3. There is no slow or rapid reaction characterizing the inbred strains as a group. The extremes of the reaction rates are represented by the Ba, slowest, and the Li, most rapid, among the inbred lines. The less highly inbred strains, the Sil and Sch, also follow the extremes of the mortality rate curves, the Sil high, and the Sch low.

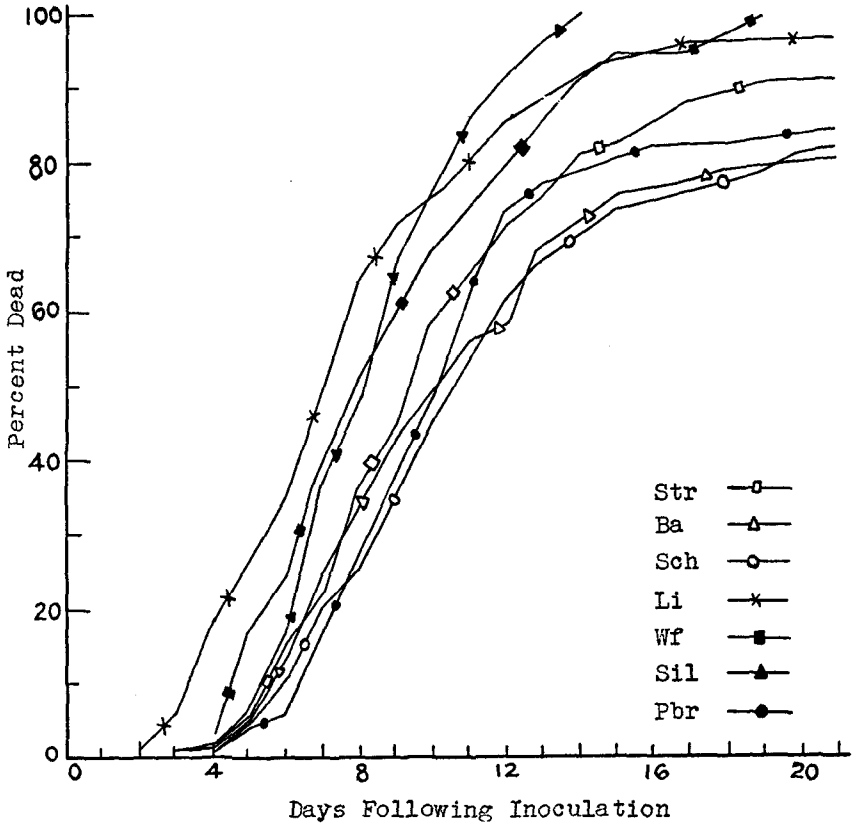

FIGURE 3.—Death rate curves of various strains of mice when inoculated with a standard dose of *S. aertrycke*, 5×10^4 organisms.

VIRULENCE OF THE ORGANISM

In studies employing living bacteria over long periods of time the question of constant virulence is most imperative. It has already been pointed out that a standard dose of 5×10^4 organisms was selected early in the course of this experiment. Throughout a period of three years 1006 mice besides those directly under selection have been tested with this standard

dose; at no test period has the organism failed to produce its quota of fatalities.

That variations do occur in cultures of *S. aertrycke* apparently spontaneously as well as under specific changes of treatment has been shown by LOCKHART (1926) and WILSON (1928, 1930, 1931). However, certain strains of the organism have been observed to maintain a high virulence over many years of culture on laboratory media. Using *S. aertrycke* and other closely allied types of paratyphoid-enteritidis organisms, WEBSTER (1923, 1924, 1925) and PRITCHETT (1926) have observed consistent pathogenicity for the mouse over long periods of time.

In summarizing his specific studies on the virulence of *S. aertrycke* WILSON (1930) states: "It appears probable that the variation in the percentage mortality following the inoculation of the control strain was due not so much to an alteration in its virulence as to fluctuations in the susceptibility of the mice."

The summary of percentage mortalities of the various strains, as given in table 5, shows that the fatalities have been high and uniformly distributed through each strain except in perhaps Pbr and Ba. In the Pbr strain the total mortality has been higher than that of the Ba or Sch strains and the apparent fluctuation from litter to litter within the strain in no way invalidates the conclusion that the organism has maintained a high and fairly consistent virulence.

RESULTS OF SELECTION FOR RESISTANCE

Selection for resistance to the disease organism has been carried on through six generations. The original progenitors for the selected group came from survivors of the Sch strain. Successive selected generations are designated as S_1S_2, etc. A summary of the selected group is given in table 6, along with the reactions of the Sch and Sil strains and a hybrid stock from Sch$\times$Sil. The stock listed as Oc represents progeny from selected resistant animals from the S_1 to S_5 mated with Silvers. Considerations of the results of reciprocal matings are made in the discussions of passive immunity and sex differences.

The S_1 animals are progeny of survivors of the Sch strain from the earlier tested groups. The S_2 stock are descendants of S_1 survivors. In the remaining selected generations occasionally an animal of S_2 was mated to S_3 or S_4, or an S_3 to an S_4 or S_5, if such matings gave promise of greater accumulation of resistance. Progeny from such crosses were recorded as of the selection above the higher numbered parent, that is, a litter from an $S_2\times S_3$ mating was recorded in the S_4 group. Since all matings were made

within closed and closely related groups, extra classification was considered as superfluous.

The groups as considered in this section furnish a general summary of the results of selection. The elements of the selection operating within the generations from the standpoint of individuals are undoubtedly of more vital interest. Their more complete analysis will be found under the section on effects of individuals on selection.

TABLE 6

Percentage mortality and type of dispersion in successive generations of stocks selected for resistance to the standard dose, 5×10^4 organisms. The Sch and Sil groups represent the total number of animals used as controls throughout the six generations.

STOCKS	NUMBER INOCULATED	NUMBER DEAD	PERCENT DEAD	LITTERS TESTED	L	X^2	P
Sil	108	108	100.0	..	..	..	..
Sch	538	443	82.3	13†	1.19	18.34	0.10
S_1	175	113	64.5	44	1.16	60.15	0.04
S_2	109	50	45.8	27	1.60	69.32	0.01
S_3	123	49	39.8	27	1.08	32.17	0.16
S_4	154	56	36.3	35	1.22	52.15	0.01
S_5	147	48	32.6	35	0.94	31.25	0.49
S_6	105	26	24.7	26	1.11	32.05	0.14
F_1 (Sch×Sil)	106	83	78.3	25	1.05	25.38	0.34
Oc (S*×Sil)	187	70	37.4	47	0.94	41.13	0.64

* Selected animals mated to Silvers.

† Groups tested.

The percentage mortalities in table 6 show a progressive increase in total resistance over the entire period of selection. The greatest effect was obtained in the first and second generations after which the progress was somewhat retarded.

Hybrids from untested Sch×Sil show a mortality approaching that of the more resistant parent strain. The dispersions of mortalities in this F_1 stock and the Sch strain are remarkably constant, being well within that expected from random variability. The high resistance in the F_1 indicates that the parental Sch strain carries inherent factors for resistance since the parental Sil strain is totally susceptible to the dosage used. No Sil animals have survived the standard dose.

In following the type of dispersion through the selected generations, it is worthy of note that in the Sch strain the probability for death is high and uniformly distributed throughout. In the successive generations, S_1, S_2, S_3, and S_4, there is apparently some disturbance of the probability ac-

companying the course of selection. The inbreeding accompanying mating in a closed population should concentrate genetic factors for resistance. If such concentrations are reflected in the tendency of a litter to react as a unit in the test then this tendency for litter differences should be reflected in the statistics describing the generation.

The S_5 and S_6 stocks with a higher concentration of factors for resistance, or, inversely stated, carrying a low probability for death, show the probability uniformly distributed from litter to litter.

The Oc stock in table 6 represents an aggregate of hybrid litters. The Sil strain was used as one parental line. The animals represented by S were from various selected generations. A comparison of the Oc stock with the F_1 stock gives emphasis to the point that genetic factors for resistance have been accumulated. The mortality in the Oc stock is almost identical with that in S_4, 37.4 percent. As yet, no S_6 animals have been mated to the Silvers. Other features of the Oc stock will be discussed under passive immunity and the effects of individuals on selection. In table 7 is given a statistical statement of the proportionate differences between variously selected stocks.

TABLE 7

Tests for independence between successive generations of the selected in addition to the control and out-cross stocks.

STOCKS COMPARED	DIFFERENCES IN PERCENTAGE MORTALITY	X^2	P
Sch and S_1	17.7	22.07	<0.01
S_1 and S_2	18.7	10.29	<0.01
S_2 and S_3	6.0	1.12	0.29
S_3 and S_4	3.5	0.55	0.46
S_4 and S_5	3.7	0.23	0.65
S_5 and S_6	7.9	1.26	0.27
S_2 and S_5	13.2	4.42	0.04
S_4 and S_6	11.6	3.63	0.06
Sil and Sch	17.7	24.50	<0.01
Sil and F_1	21.7	17.58	<0.01
Sch and F_1	4.0	0.66	0.44
Sch and Oc	45.9	134.05	<0.01
Sil and Oc	62.6	112.45	<0.01
F_1 and Oc	40.9	53.20	<0.01

The differences between Sch and S_1, and S_1 and S_2 are statistically significant, indicating that causes other than chance were operating to produce these differences. Taken consecutively from S_2 to S_6 any single interval represents differences easily attributable to variation of random

sampling. However, between S_2 and S_5, and between S_4 and S_6 the differences approach a significant figure supporting the conclusion that over the series of generations the selection has been effective in increasing the level of inherent resistance.

Considering Sch, Sil and their F_1 progeny the difference between the parental strains is significant; between Sil and F_1 is seen statistical identity

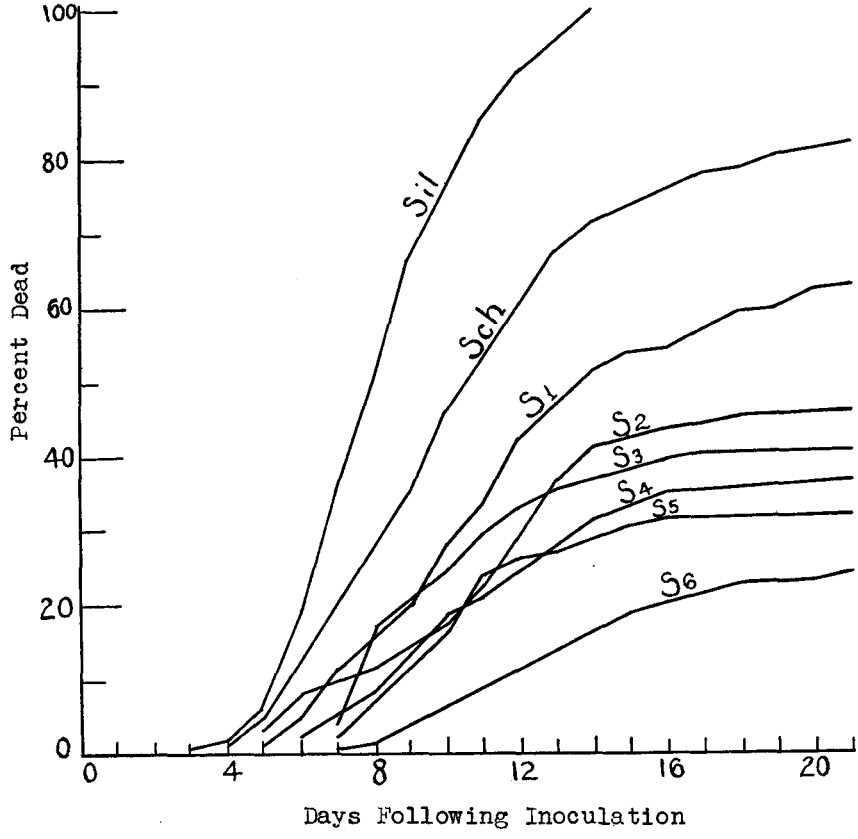

FIGURE 4.—Comparison of death rates of stocks selected for resistance; inoculation with standard dose 5×10^4 organisms.

in their proportionate mortality. The Oc stock shows complete statistical independence from the above three stocks. In each case the P is well beyond 0.01.

A comparison of the percentage mortality rates among the selected stocks is given in figure 4.

The curves show much the same rate of mortality from the S_2 to the S_5

generations. The S_6 group gives a somewhat retarded rate of death, but in order to attach significance to that it would require tests on much larger numbers of similarly selected animals.

In figure 5 is given a comparison of the percentage mortality rates of the F_1 and Oc stocks along with the rates of the Sil and Sch strains.

The similarity of the F_1 reaction to that of the Sch is very striking, again

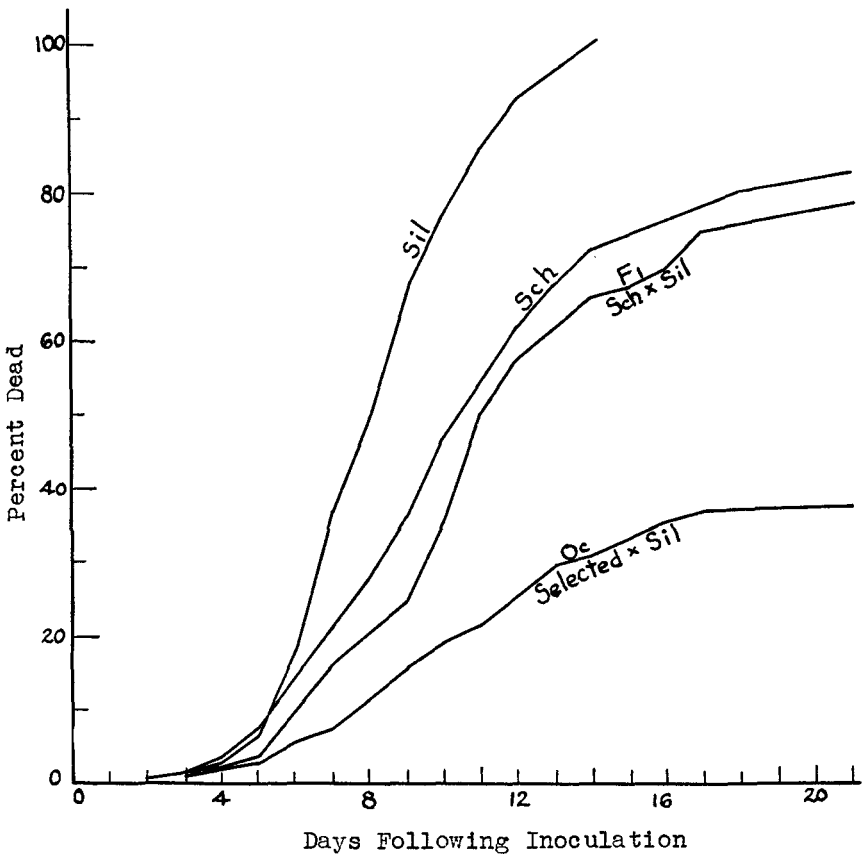

FIGURE 5.—Mortality rates in Sil, Sch, F_1 (progeny of Sil×Sch), and Oc (selected resistant animals×Sil) stocks under the standard dose, 5×10^4 organisms.

lending support to the belief that the Sch stock carries dominant, genetic factors for resistance. The wide separation between the F_1 curve and that of the Sil parental strain also supports the conclusion drawn from data in table 6 that the F_1 progeny inherited their resistance from the Sch parental strain.

The reaction ot the Oc stock, as shown by its mortality curve, has little

in common with that of the Sil parental strain. Its analog is found among the mortality curves of the selected generations in figure 4. The point to be emphasized is that certain individuals after selection for resistance when mated to totally susceptible animals can produce progeny that resist the disease as completely as progeny from matings among the selected animals themselves.

The conclusion that a complex of at least partially dominant genes is largely responsible for resistance would seem justified from the facts that: (1) unselected animals from the Sch strain when mated to Silvers produce progeny practically as resistant as the Sch animals, and (2) selected resistant animals when mated to Silvers produce progeny as resistant as the fourth surviving generation. The fifth surviving generation continues to throw a considerable number of susceptible animals. This indicates that the selected population is still carrying some factors for susceptibility.

PASSIVE IMMUNITY

If passive immunity were considered as largely responsible for the increased resistance resulting from selection, the observations in this experiment could not be used to support this assumption. Males in mammals are not known to be able to transmit acquired immunity to their progeny. If males surviving the disease are mated to females that are from known susceptible stock, and these females themselves have had no direct contact with the disease, the progeny from these matings should be largely susceptible. A part of the Oc stock was made up of progeny from such matings. At test the following results were obtained:

Number tested	*Number dead*	*Percent dead*
160	60	37.5

The argument for passive immunity in this case is invalid, for the resistance in the 160 Oc mice is practically equivalent to that in the S_4 selected resistant generation, that is, 36.3 percent.

If a latent infection capable of rendering mice highly resistant were at large in the propagation laboratory at any time during the three years of the experiment, it would seem remarkable that some animals of the Sil or Wf strains had not acquired the protection before coming to test.

Furthermore, four Sil females, each having first produced an Oc litter, were mated to Sil males. Fourteen of the Sil offspring from these matings came to test and none showed indifference to the infection. A comparison of the progeny of the four Sil females shows the following:

Stock	*Number tested*	*Number dead*	*Percent dead*
Oc	17	6	35.39
Sil	14	14	100.00

If passive immunity were operating, the progeny from selected resistant females on Sil males should have a marked advantage over the progeny from the reciprocal matings. Progeny from six selected females mated to Sil males were tested, and gave the following reaction:

Number tested	*Number dead*	*Percent dead*
27	10	37.0

While the number in this group is too small to carry much weight, the reaction suggests no real advantage in resistance over the progeny from the reciprocal crosses.

The fact that the young mice in all cases were separated from their mothers approximately four weeks before testing would seem to be sufficient ground for believing that any temporary passive immunity derived from the mother had been lost before the time of inoculation.

From the above considerations it is concluded that passive immunity has played a minor, if any, part in the resistance observed in this study.

AGE, WEIGHT, AND SEX AS FACTORS IN RESISTANCE

Experience with 2901 mice at test precludes the assumption that age within limits concerned in this study, or weight as incidental to age, or sex, has played any critical part in resistance. A summary of these features relative to certain salient groups is given in table 8.

The S_1 generation was chosen as an example of the early stages of the selection and the S_5 is representative of the advanced selection. In the S_1 males as shown in table 8 there is an apparent tendency for a higher percentage of the younger and lighter males to die. This is not apparent in the corresponding female group, however.

TABLE 8

Showing average age, weight, and days to death in S_1 and S_5 male and female groups.

STOCK	SEX	NUMBER	AVERAGE AGE IN DAYS	AVERAGE WEIGHT IN GRAMS	AVERAGE DAYS TO DEATH
S_1	♂♂	Survive 31	60.3±0.64	19.2±0.31	..
		Dead 60	56.7±0.45	18.4±0.17	11.8±0.28
	♀♀	Survive 31	58.4±0.66	16.7±0.24	..
		Dead 53	58.5±0.75	16.5±0.18	11.1±0.46
S_5	♂♂	Survive 49	49.9±0.94	18.9±0.18	..
		Dead 23	62.8±0.60	19.1±0.26	11.4±0.47
	♀♀	Survive 50	60.8±0.47	16.5±0.14	..
		Dead 25	60.7±1.00	17.2±0.19	10.8±0.39

In the S_5 stock the apparent differences in S_1 are reversed. Neither in these two groups nor in any other of the six selected generations was there any marked advantage in resistance noted because of greater age or weight.

Neither has sex given any indication of association with resistance in any group. In the S_1 and S_5 generations the proportionate mortalities in the sex groups are practically identical.

In the Sil stock where, presumably, factors for resistance are absent the effects of age and weight can be spoken of only as they are associated with rate of mortality. The correlation coefficient between age and days to death is 0.017 and that between weight and days to death is 0.299. Between weight and age there is an r of 0.134. If one refers to FISHER's (1930, p. 176) table for significance of correlation coefficients, the r of 0.299 is significant. The heavier animals in the Sil strain tend to live slightly longer than the light ones.

Among the Oc animals which were sired by selected males it might be expected, since the male is heterozygous for sex, that the association of resistance with maleness or femaleness would have opportunity of expression. The 160 Oc mice from selected resistant sires show:

	Number tested	*Number dead*	*Percent dead*	*Percent difference*	X^2	*P*
♂♂	87	31	35.6	4.1	0.43	0.65
♀♀	73	29	39.7			

The proportionate difference in mortality between males and females in this group is too small to suggest any advantage of one sex over the other.

INBREEDING IN THE SELECTED GROUPS

Mating within a closed population from generation to generation should automatically increase the homozygosity of the group. The inbreeding and relationship coefficients in the selected generations have been calculated according to the method of WRIGHT (1923).

The surviving animals from the earlier tests on the Sch strain were mated together. Their surviving progeny in turn were intermated, and the whole series of related animals through six generations was designated as group I. Another similar series, group II, was developed from Sch survivors that were at test later than the progenitors of group I. Group II was also mated entirely within itself and includes animals from the S_1 to S_5 generations.

The percentage mortality in group I in each consecutive generation with the average inbreeding is given in table 9. The percentage dead from generation to generation does not decrease uniformly, while the inbreeding increases at a quite uniform rate.

TABLE 9

Percentage mortality and percent inbreeding per generation in group I.

GENERATION	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	AVERAGE PERCENTAGE INBREEDING
S_1	30	16	53.3	0.0
S_2	46	19	41.3	14.2
S_3	65	30	46.0	23.9
S_4	95	31	31.5	31.5
S_5	119	38	31.9	33.3
S_6	105	26	24.7	43.0

The distribution of mortalities in each generation relative to the amount of inbreeding is listed in table 10. Here it will be noted that there is a considerable range of inbreeding in each generation after the S_1.

TABLE 10

Generations in group I showing percentage mortality for each ten percent range of inbreeding.

PERCENT INBREEDING	S_1		S_2		S_3		S_4		S_5		S_6	
	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED
50–59	..	..	..	..	..	..	..	..	..	..	35.9	(26)
40–49	..	..	..	..	75.0	(4)	37.0	(27)	26.8	(41)	19.5	(46)
30–39	..	..	28.5	(14)	40.0	(10)	25.0	(28)	31.5	(38)	23.9	(17)
20–29	..	..	..	..	41.4	(41)	56.2	(16)	40.0	(15)	37.5	(16)
10–19	..	..	42.8	(14)	12.2	(7)	26.3	(24)	36.0	(25)	..	..
0– 9	53.3	(30)	50.0	(18)	100.0	(3)	..	..	..	..	..	..

In the second series of matings designated as group II the same general trends and relationships are apparent as were noted in group I. The summaries of the group II percentages are given in tables 11 and 12.

TABLE 11

Percentage mortality and percentage inbreeding in the generations of group II.

GENERATION	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	AVERAGE INBREEDING
S_1	37	19	51.0	0.0
S_2	63	31	49.2	8.3
S_3	58	19	32.7	23.9
S_4	59	25	42.4	32.8
S_5	28	10	35.7	29.8

In table 12, as was pointed out in table 10, there is considerable variation in the inbreeding of any one generation.

TABLE 12

Distribution of inbreeding in consecutive generations relative to mortality in the generations of group II.

PERCENT INBREEDING	S_1		S_2		S_3		S_4		S_5	
	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED	PERCENT DEAD	NUMBER INOCULATED
50–59	..	..	..	..	..	..	..	..	33.3	(3)
40–49	..	..	..	..	..	..	33.3	(6)	..	..
30–39	..	..	..	..	38.4	(13)	38.4	(26)	..	..
20–29	..	..	..	..	31.1	(45)	48.1	(27)	36.0	(25)
10–19	..	..	42.8	(42)	..	..	..	..	..	..
0– 9	51.6	(37)	61.9	(21)	..	..	..	..	..	..

If there were a tendency for higher inbreeding as such to be associated with lower resistance the higher mortality percentages would be distributed through the upper ranges of the tables. This or the inverse situation is not apparent. Therefore, it must be concluded that the amount of inbreeding as expressed by the coefficients is no direct index of resistance or susceptibility in the animals used in this experiment. However, it is believed that the inbreeding and the relationship incidental to it are useful descriptive measures in the analysis of the uniformity of any group of animals.

The slight fall in the average inbreeding in the fifth generation of group II as shown by table 11 indicates only that the less highly inbred animals of the preceding generation have thus far had progeny at test.

The whole upward trend of the inbreeding coefficients in the two groups gives a fair picture of the gradual tendency toward homozygosity. If the resistance were heightened by homozygous factors then the course of selection should have increased the homozygosity above that of the average estimated figure. If selection were favoring the heterozygous individuals, on the other hand, then the average estimated coefficient of inbreeding for any generation would be higher than the actual homozygosity in that generation.

As yet there is no means of measuring the whole genetic make up of an animal. However, by a gradual process of narrowing the chances for heterozygosity by breeding within a closed group, along with rigid selection, there should be a tendency toward a more uniformly resistant population. The selection, however, is incomplete if the individual's ability to survive

is the only criterion used. The manner in which individual performance coupled with that of progeny and progenitors has been used in the selection will be pointed out below.

THE INFLUENCE OF INDIVIDUALS IN SELECTION

Among the Sch survivors that were the parents of the S_1 generation in group I, a male, Sch 162, and a female, Sch 217, were from separate sire-daughter matings. This male and female were 25 percent inbred and not more closely related than the average individuals of the Sch strain. The reactions of the progeny of these two individuals supported the belief that already a considerable concentration of factors for resistance had taken place. Their progeny predominate in the S_1 generation. Only four unrelated S_1 females were mated into the group to produce the S_2 generation.

Three S_1 males, $S_1$1, $S_1$3, $S_1$8, all brothers, from ♂ Sch 162 × ♀ Sch 217, sired the entire S_2 generation of group I. A summary of the reaction of the progeny of each of these males is given in table 13 along with the average inbreeding coefficient of each progeny group under F_o and the average relationship coefficient of the sire to his progeny under R_{so}.

TABLE 13

Percentage mortality of progeny of S_1 males with average inbreeding and average relationship coefficients.

SIRE	DAMS	NUMBER INOCULATED	PERCENT DEAD	F_o AVERAGE INBREEDING	R_{so} AVERAGE RELATIONSHIP OF SIRE AND PROGENY
$S_1$1	Selected ♀♀	21	33.3	19.2	60.8
	Sil ♀♀	5	40.0	0.0	50.0
$S_1$3	Selected ♀♀	14	57.1	8.9	56.1
	Sil ♀♀	7	28.6	0.0	50.0
$S_1$8	Selected ♀♀	11	36.4	11.4	57.6

The data in table 13 show that there was some homozygosity possible in the S_2 progeny although the S_1 parents were not inbred. The homozygosity came from the two inbred grandparents by brother-sister and half brother-sister matings.

There were three litters in the S_2 generation from brother-sister matings, one from each of the S_1 males. The 14 young in the three litters were 31.25 percent inbred. This rapid concentration of factors seemed not to hinder resistance for 10 of the 14 survived the test. However, of the five survivors

of the litter of 8 sired by ♂ $S_1$1, two males were discarded at the close of the test period and the remaining two males and one female proved sterile in all matings. The remaining two litters consisted of a male and female sired by $S_1$3, and one male and three females sired by $S_1$8. The two individuals from ♂ $S_1$3 were designated as ♂ $S_2$87 and ♀ $S_2$88. Both survived the test. Of those from ♂ $S_1$8 the three females survived. Male $S_2$87 became the dominating sire of the following generations in group I through the good resistance of this progeny and the favor which certain of them received in selection.

That certain animals can resist the disease and yet be of no use in the accumulation of resistance can be illustrated by the performance of progeny from ♂ $S_3$8. When mated to ♀ $S_3$14 he gave a litter of four. All four died at test. At the same time he produced two litters by Sil females, 9 Oc progeny. Eight of these died at test.

It was decided to make further tests of the apparent susceptibility of the progeny of ♂$S_3$8 and ♀ $S_3$14. A second litter was produced, one male and three females. Before these were mature the sire had died so the litter was not tested. The dam and three daughters were mated to the young male, and progeny coming to test gave:

Number tested	*Number dead*	*Percent dead*
28	19	67.8

Three males and nine females of the brood of ♀ $S_3$14 were not tested; matings among this group gave:

Number tested	*Number dead*	*Percent dead*
27	17	62.9

These results of negative selection show that although some individuals can resist the disease they carry heritable factors that tend to render some of their progeny highly susceptible. It would be presumptious to suppose that all of the possibilities for detrimental combinations had been eliminated from the resistant animals after three or even six generations of selection.

That male $S_2$87 carried high potentialities for heritable resistance can be concluded from the list of his progeny given in table 14.

It will be noted that resistance in the progeny by S_3 females is better than that of the progeny by the S_2 females. Also, the inbreeding of the progeny and the relationship of the progeny to the sire have increased. Stated in terms of mean percentages these show:

Dams of generation	*Number of progeny tested*	*Percent dead*	*Average F_o*	*Average R_{so}*
S_2	18	50.0	31.6	74.5
S_3	28	25.0	41.5	77.9

Perhaps no better description of the importance of ♂ $S_2$87 in the group I population can be given than by noting the increase of his average relationship to each consecutive generation. The coefficients are given in table 15 along with the relationship of a male from each generation having the highest coefficient other than ♂ $S_2$87. The male listed in each case is

TABLE 14

Progeny of ♂ $S_2$87 from various females showing number dead, percent inbreeding, and relation of progeny to the sire.

DAMS	NUMBER TESTED	NUMBER DEAD	F_o PERCENT INBREEDING OF PROGENY	R_{so} RELATIONSHIP OF SIRE TO PROGENY
$S_2$74	4	0	23.4	69.9
$S_2$92	2	2	31.2	73.8
$S_2$91	8	4	31.2	73.8
$S_2$88	4	3	40.6	80.9
$S_3$20	11	0	32.0	74.2
$S_3$10	3	3	44.5	74.2
$S_3$42	6	2	48.4	81.7
$S_3$43	8	2	48.4	81.7
$S_4$4	7	3	38.3	77.2
Sil ♀ ♀	16	5	00.0	50.0

the one having the most offspring in the following generation. Also the relation between the listed male and ♂ $S_2$87 is given. The coefficients of relationship between males and generations in the table are in all cases relative to the group of individuals which produced progeny and not to the generation as a whole.

The relationship coefficients of ♂ $S_2$87 to the reproducing individuals show more than a doubling from the S_1 to the S_5 generation. This increase in the coefficient values indicates that factors carried by ♂ $S_2$87 have become 67.5 percent of all the factors carried by the reproducing individuals in the S_5 generation.

The males from consecutive generations as listed in table 15 were all more closely related to ♂ $S_2$87 than brothers in a random bred population. Male $S_4$5 was a son of ♂ $S_2$87 × ♀ $S_3$20. He sired eleven of the twenty-seven litters in the S_5 generation group.

TABLE 15

The relationship of ♂ $S_2$87 to the reproducing group in five generations, and the relationship between certain males to their own generation and to ♂ $S_2$87.

GENERATION*	S_1	S_2	S_3	S_4	S_5
Average R of generation to ♂ $S_2$87	32.2	42.8	51.6	62.7	67.5
Selected sire of each generation	♂ $S_1$1	♂ $S_2$69	♂ $S_3$31	♂ $S_4$5	♂ $S_5$3
Selected sires' relationship to his own generation	42.7	28.9	37.6	51.7	62.3
Selected sires' relationship to ♂ $S_2$87	54.5	35.4	36.1	57.7	70.1

* The relationship of any sire to the generation refers to that group of individuals which produced progeny.

It was evident that certain S_3 litters, closely related to ♂ $S_2$87, were at an advantage in the tests. Matings in the fourth and fifth generation, therefore, were made to favor a concentration of factors carried by individuals from such litters. This concentration of factors is reflected in the increased relationship of ♂ $S_2$87 to the S_5 over his relationship to the S_4. He had sired only one litter listed under S_5 while he had sired five litters listed under S_4.

Examination of the relationships of ♂ $S_2$87 shows: ♂ $S_4$5 mated to a sister, ♀ $S_4$6, produced two S_5 litters, and with another sister, ♀ $S_4$8, he gave one litter. Of the 13 progeny, 10 survived. Male $S_5$3 listed in table 15 was from this group. Male $S_5$4 was a full brother of ♂ $S_5$3 and ♂ $S_5$1 was from ♂ $S_4$5 × ♀ $S_4$6. The three S_5 males, $S_5$1, $S_5$3, and $S_5$4, sired 14 of the 26 litters of the S_6 generation that have been tested. This inbred line of descendants kept a high concentration of the factors of ♂ $S_2$87 in the S_6 generation. The average coefficient of relationship between ♂ $S_2$87 and the S_6 generation is 51.5 percent. This indicates that although he sired none of the S_6 animals he still had the genetic status of sire to that generation.

It is believed that through the detection of the inherent potentialities for resistance in ♂ $S_2$87 and through the subsequent concentration of those potentialities it has been possible to maintain and increase the resistance through the later generations. Continued inbreeding in a naturally cross-bred species is generally considered as a precarious practice with no other demands on the individuals than reproduction. In this study the additional requirements have been ability of the animal to resist the disease as well as to transmit the factors that make for resistance. The conclusion seems justifiable that animals having these requirements have been found and that concentration of the underlying heritable factors has been carried on as rapidly as was allowable with the population at hand.

In group II the same tendency for the generations to follow the line of certain males is evident. A detailed analysis of group II would be a needless repetition of the methods explained above. The increase of the inbreeding coefficients as shown in table 11 implies that there has been a gradual increase of the relationship between individuals, and a gradual concentration of factors for resistance has been carried on by mating progeny from individuals that have proved their worth by giving a high percentage of resistant offspring.

SUMMARY

In this investigation a total of 2901 mice have been inoculated intraperitoneally with controlled doses of living *Salmonella aertrycke.*

A standard dose of 5×10^4 organisms was used as a basis of selection for resistance after the reactions of three different laboratory strains of mice had been observed under various sized doses. Animals from four other distinct strains of mice have been tested with the standard dose.

The seven strains of mice and their respective percentage mortalities under the standard dose were: Schwing albino (82.3), Bagg albino (80.4), Strong dark-brown (90.7), Little dilute-brown (96.4), Short-eared dilute-brown (84.7), English silver (100.0), and White-faced (100.0).

Selective breeding for resistance was continued through six successive generations using survivors of the Schwing strain as the beginning of the selection. The percentage mortality in the successive selected generations was as follows: Schwing (82.3), first (64.3), second (45.8), third (39.8), fourth (36.3), fifth (32.6), and sixth (24.7).

Untested Schwing mice were mated to Silvers and the F_1 progeny at test gave a mortality percentage of 78.3. Selected resistant animals mated to Silvers gave progeny which showed only 37.4 percent dead at test. These results with the hybrid generations together with a consideration of the progressive increase in resistance in the six selected generations justify the conclusions: a marked concentration of heritable factors for resistance has been obtained; this resistance depends largely on a complex of at least partially dominant genetic factors.

It is shown that the possible effects of passive immunity as an explanation of increased resistance could be of no importance in the resistance observed.

Within the limits concerned in this study, age at the time of testing and likewise weight have not played a critical part in resistance. One sex shows no advantage over the other at test.

Coefficients of inbreeding and relationships have been calculated for the selected groups and used in the report as a descriptive estimate of genetic uniformity.

It has been pointed out that phenotypic resistance of the individual animal alone cannot be used as the only criterion for selection. An intimate knowledge of the individuals in relation to their progenitors and progeny is necessary in order to make matings that will maintain and increase the high level of resistance over a series of generations in a closed population.

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