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GENETICAL STUDIES ON IMMUNITY IN MICE

II. CORRELATION BETWEEN ANTIBODY FORMATION AND RESISTANCE

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AT the present time there is little reason for doubting the existence of genetic differences affecting resistance to infection in mice (see review by Bradford Hill (1934), and papers by Webster (1937), Schütze *et al.* (1936) and Hetzler (1937)).

It seems desirable at this point to see whether the agglutinin-producing capacity of a mouse strain can be correlated in any way with the observed resistance of that strain. The only studies of this kind made up to the present time appear to be those of MacMaster & Hudack (1934), who tested the agglutinin titres of pooled extracts of lymph nodes and of pooled sera from Webster's susceptible and resistant strains. Results of this type give no idea of the variability within the strains and thus cannot be compared with those to be given below. A further point that needs investigation is the extent to which active immunization can bridge the gap between strains of varying resistance.

To investigate these points use was made of strains that were known to differ from one another in their resistance to certain *Salmonella* infections. Two of these strains had been selected by Webster (1933) for resistance and susceptibility to *S. enteritidis* and are known in this laboratory as strains "A" and "B" respectively; they have not been closely inbred. In addition two highly inbred strains were used which have been shown (Schütze *et al.* 1936) to differ in their response to *Salmonella* infections. They are Strong's A_2 strain (strain D in this laboratory) and Little's strain C 57 Blacks (strain E of this laboratory, previously referred to inaccurately as Dunn's blacks). These two strains have been brother-sister inbred for over thirty generations, and it is customary to refer to such strains as pure lines. Although Haldane (1936) has pointed out that this term is probably never more than approximately true, experience has shown that the approximation is sufficiently close for many purposes.

The primary object of the present study is the detection of genetic differences between the four strains of mice, but in order to do this it is necessary to assess the causes of variation within a strain. A possible cause of this type of variation may be attributed to sex. Sex differences are found in a large number of biological reactions (Gaddum, 1933; Gowen, 1936) and have been observed in susceptibility to infection with mouse typhoid by Schütze *et al.*

(1936) and Hetzler (1937). This factor has been taken into account in the assessment of results to be given below.

The statistical examination of the results was kindly undertaken by Dr P. S. Hsu working in the Department of Applied Statistics, University College, under the direction of Dr J. Neyman. The methods are given by Neyman (1937) and Neyman & Johnson (1936).

When last tested for resistance to *S. typhi murium* the four strains fell into the following order of diminishing resistance: $A > D > \frac{B}{E}$.[1] The females appeared more resistant than the males. It is possible in the last three groups that this order might not be found on every occasion. It is very unlikely that the two pure lines (D and E) would change much, but, as will be shown later with *S. enteritidis*, the B group is certainly not homogeneous for genes affecting resistance. If, as appears likely on certain grounds (Hill, 1934; Webster, 1937), there are a small number of genes having a marked effect on resistance, the mean expectation of life in a genetically heterogeneous group might show significant changes from sample to sample along with a corresponding alteration in gene frequencies. In spite of the doubtful value of the "B" group it is probably safe to accept the order given above for the other groups.

"H" AGGLUTININ PRODUCTION AGAINST *S. TYPHI MURIUM*

To test the ability of the four groups to form antibodies about twenty mice from each group received three subcutaneous injections of a heat-killed (70 min. at 53° C.) *S. typhi murium* vaccine which had phenol added as a preservative, the successive doses being 200, 400 and 400 millions respectively. A week after the last injection the animals were bled by snipping the tail and tested for "H" and "O" agglutinins. It is generally possible to obtain about 0·25 c.c. of serum if the animals are warmed prior to bleeding.

In assessing the results of agglutination experiments it is necessary to calculate the geometric mean titre as the arithmetical average is devoid of significance. The most convenient way of calculating the mean is to assign the numbers 1, 2, 3, etc., to the different dilutions and take the average of these figures. For instance, in Table I*a*, as tube 1 contained a 1 : 50 serum dilution, tube 2 would have 1 : 100, tube 3 1 : 200 and so on. Therefore tube 5 contained 1 : 800 and the mean titre for A ♀ mice being expressed by the factor 5·25, would be 25 % in excess of 1 : 800, viz. 1 : 1000; and the mean titre for B ♀ mice (3·75) would be 75 % in excess of 1 : 200, viz. 1 : 350. By reckoning in this way from the tube 1 serum dilution, as given in the footnote to each table, the actual titres can be obtained.

The results obtained for "H" agglutinins are shown in Tables I*a*–I*e*, and those for "O" agglutinins in Tables II*a*–II*e*.

[1] The placing of B above E in this fashion indicates equality.

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Considering the sexual differences in "H" antibody production (Tables I*a* and I*b*) one can see that the females react better in all groups, but the difference is significant in the A and E groups only. If the data for all four groups are pooled, the difference between females and males is 0·68 unit with a standard

Table I*a*. *Mean titres for males and females of different strains*

	Mouse strain							
	A		B		D		E	
Sex ...	♂	♀	♂	♀	♂	♀	♂	♀
Mean titre	4·16	5·25	3·18	3·75	4·00	4·45	3·04	3·63
No. of mice	24	20	22	20	23	20	25	19
Estimated S.E.	0·27	0·22	0·23	0·27	0·20	0·33	0·18	0·16

S.E. = standard error. Tube 1 = serum dilution 1 : 50.

Table I*b*. *Difference between the sexes of the same strains*

	Mouse strain			
	A	B	D	E
Estimated difference	1·08	0·57	0·45	0·59
Estimated S.E.	0·36	0·39	0·38	0·25

Table I*c*. *Difference between males of different strains*

	Mouse strains					
	A and B	A and B	A and E	B and D	B and E	D and E
Estimated difference	0·99	0·17	1·13	−0·82	0·14	0·96
Estimated S.E.	0·33	0·33	0·32	0·34	0·33	0·33

Table I*d*. *Difference between females of different strains*

	Mouse strains					
	A and B	A and D	A and E	B and D	B and E	D and E
Estimated difference	1·50	0·80	1·62	−0·70	0·12	0·82
Estimated S.E.	0·36	0·36	0·36	0·36	0·36	0·36

Table I*e*. *Comparison of strains regardless of sex*

	Mouse strains					
	A and B	A and D	A and E	B and D	B and E*	D and E
Estimated difference	1·22	0·46	1·35	−0·76	0·13	0·89
Estimated S.E.	0·24	0·24	0·24	0·25	0·25	0·24

* For B and E, $t = 0{\cdot}52$ and is not significant. All others give significant values for t.

error of 0·17. It seems justifiable to conclude that females give higher "H" agglutinin titres than males.

When the ability of the different lines to produce "H" antibodies are compared, Tables I*c* and I*d* show that the inter-line differences are not equally well known by both sexes. For example, in the males it is not possible to differentiate the A and D groups, whilst in the females the difference

between these two groups is significant. On the other hand the reverse is true when the B and D groups are compared, for here the difference is significant for males and not for females. The B and E groups do not appear to differ significantly. Whichever sex is used the lines fall into the same order although the differences are not equally significant in both sexes. Similarly, if the lines

Table II*a*. *Mean titres for males and females of different strains*

	Mouse strain							
	A		B		D		E	
Sex ...	♂	♀	♂	♀	♂	♀	♂	♀
Mean titre	2·56	3·00	2·48	3·45	3·39	4·00	3·12	3·95
No. of mice	23	20	23	20	23	20	25	19
Estimated S.E.	0·21	0·31	0·23	0·34	0·17	0·28	0·19	0·35

Tube 1 = serum dilution 1 : 12·5.

Table II*b*. *Difference between the sexes of the same strains*

	Mouse strain			
	A	B	D	E
Estimated difference	0·43	0·97	0·61	0·83
Estimated S.E.	0·36	0·40	0·32	0·37

Table II*c*. *Difference between males of different strains*

	Mouse strains					
	A and B	A and D	A and E	B and D	B and E	D and E
Estimated difference	0·09	−0·82	−0·55	−0·91	−0·64	0·27
Estimated S.E.	0·35	0·35	0·33	0·35	0·33	0·33

Table II*d*. *Difference between females of different strains*

	Mouse strains					
	A and B	A and D	A and E	B and D	B and E	D and E
Estimated difference	−0·45	−1·00	−0·95	−0·55	−0·50	0·05
Estimated S.E.	0·38	0·38	0·38	0·38	0·38	0·38

Table II*e*. *Comparison of strains regardless of sex*

	Mouse strains					
	A and B*	A and D	A and E	B and D	B and E	D and E*
Estimated difference	0·16	−0·90	−0·73	−0·74	−0·57	0·17
Estimated S.E.	0·26	0·26	0·25	0·26	0·25	0·25

* For A and B, and D and E, $t = 0{\cdot}61$ and 0·68 respectively, and is not significant. All others are significant.

are compared regardless of sex (Table I*e*) the order is $A > D > \frac{B}{E}$, and it must be noted that the mean titres fall in the same order as do the resistances.

"O" AGGLUTININ PRODUCTION AGAINST *S. TYPHI MURIUM*

In the case of "O" antibodies, females give significantly higher titres than the males in the B, D and E groups (Tables II*a* and II*b*), whilst with the pooled data the difference is 0·71 with a standard error of 0·18 which is, therefore, highly significant.

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If the lines are compared for "O" antibody production (Tables II*c* and II*d*) we find, as before, that the differentiation is not equally clear with both sexes. It will be seen (see also Table II*e*) that the pure lines D and E are indistinguishable from each other but give higher titres than the selected stocks A and B which are also virtually equal.

AGGLUTINATION PRODUCTION AND RESISTANCE IN THE *TYPHI MURIUM* INFECTION

These data show that female mice give higher titres than males for both "H" and "O" agglutinins against *S. typhi murium*. It would also appear that the genes determining the degree of natural resistance are not identical with those influencing the ability to produce higher titres of "O" antibody. On the other hand it is possible that some of the genes affecting natural resistance may also tend towards the production of higher titres of "H" antibody. However, the latter statement should be treated with the greatest reserve. Suppose, for example, that conclusions concerning "O" antibody production had been based on the available information concerning the B and D groups, we should have obtained support for the hypothesis that natural resistance and the ability to produce antibody are positively correlated. On the other hand, a comparison of the A and E groups would have led to the conclusion that these two properties were negatively correlated. As has been mentioned earlier, data concerning the B group must be treated with reserve but a comparison of the A, D and E groups for "O" antibody production and resistance to *S. typhi murium* definitely excludes a positive correlation between the two properties, whilst the existence of a strong negative correlation is not favoured by a comparison of the D and E groups.

I. "O" AGGLUTININ PRODUCTION AGAINST *S. ENTERITIDIS*

(*a*) *Normal "O" agglutinins*

In dealing with *S. enteritidis* use was made of the well-known fact that it shares an "O" antigen with *S. typhi* and as there exists a highly agglutinable strain (O 901) of the latter organism, it was used in the two following experiments.

By using this highly sensitive strain, it was possible to detect normal agglutinins in the sera of animals from all four groups. The results are shown in Tables III*a* and III*b*.

Table III*a*. *Mean titres for males and females of different strains*

	Mouse strain							
	A		B		D		E	
Sex ...	♂	♀	♂	♀	♂	♀	♂	♀
Mean titre	2·80	2·40	3·70	3·50	4·30	4·10	5·00	4·40
No. of mice	10	9	10	10	10	10	11	9
Estimated S.E.	0·44	0·44	0·39	0·31	0·15	0·23	0·26	0·17

Tube 1 = serum dilution 1 : 2·5.

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Table III*b*. *Comparison of strains regardless of sex*

	Mouse strains					
	A and B	A and D	A and E	B and D	B and E	D and E*
Estimated difference	−0·97	−1·57	−2·12	−0·60	−1·15	−0·55
Estimated S.E.	0·31	0·31	0·31	0·30	0·30	0·30

* For D and E, $t = -1{\cdot}83$; $p > 0{\cdot}05$. In all other cases $t > 2$.

It will be seen that the males tend to give a slightly higher titre than females, but only in the E group is this difference significant (difference = 0·5; standard error = 0·22). Even if the data from all four groups are pooled no significant sex difference is to be found; therefore, it seems permissible to compare the lines as a whole. As can be seen from Table III*b*, the lines fall into the following order, E > D > B > A. The difference between the E and D groups is on the verge of significance and a larger sample might well have given a clear differentiation. The order of diminishing resistance against *S. enteritidis* established for these strains in a previous paper (Schütze *et al.* 1936) being $A > \frac{B}{D} > E$, this result shows clearly that there is no positive correlation between the titre of normal "O 901" antibody and natural resistance; on the other hand, there is a suggestion of a negative correlation.

(*b*) *Immune "O" agglutinins*

In the first experiment for the investigation of antibody production after inoculation with *S. enteritidis*, as there were insufficient unused line mice available, the mice already used in the foregoing experiment with *S. typhi murium* were again injected, this time with an *enteritidis* strain, the doses being 100, 400 and 400 millions at weekly intervals. The mice were bled one week after the last injection and the sera tested against "O 901". The results are shown in Tables IV*a* and IV*b*.

Table IV*a*. *Mean titres for males and females of different strains*

	Mouse strain							
	A		B		D		E	
Sex ...	♂	♀	♂	♀	♂	♀	♂	♀
Mean titre	1·86	2·11	2·61	2·45	3·71	3·10	3·62	3·67
No. of mice	14	18	18	20	24	20	24	18
Estimated S.E.	0·27	0·37	0·39	0·35	0·35	0·40	0·24	0·24

Tube 1 = serum dilution 1 : 25.

Table IV*b*. *Comparison of strains regardless of sex*

	Mouse strains					
	A and B*	A and D	A and E	B and D	B and E	D and E*
Estimated difference	−0·53	−1·43	−1·64	−0·90	−1·11	−0·21
Estimated S.E.	0·35	0·34	0·35	0·33	0·33	0·32

* For A and B, $t = -1{\cdot}51$, $p > 0{\cdot}1$; for D and E, $t = -0{\cdot}66$.

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No significant differences in antibody response attributable to sex could be discovered. When the strains are compared, it will be seen that the D and E groups are not distinguishable but tend to give a considerably better response than the A and B groups, themselves also virtually indistinguishable. It will be seen that the result of this experiment is similar to that obtained with *S. typhi murium* "O" antibody.

II. THE FORMATION OF *S ENTERITIDIS* "H" AND "O" ANTIBODIES

Two further *enteritidis* experiments were now carried out. The scheme of immunization was the same as previously, the sera being tested one week after the last injection.

In the first experiment only strains B and D were available; the number used may be seen in Table V*a*. In the second experiment all four lines were used but there was a considerable shortage of animals from strain A, only sixteen females being available. It will be noted that this time "O" antibody is demonstrated by agglutination with the non-motile *enteritidis* strain of Felix OGI. The reason for this change was the discovery that O 901 was not specially sensitive to agglutination by sera from immunized mice. It was shown by Felix (1930) that the O 901 strain gave a titre four times that obtainable with other typhoid or *enteritidis* strains when tested with rabbit, horse or human sera. This superior sensitivity appeared to be present in the experiments with normal mouse sera reported above. But in the case of sera from immunized mice, rats or guinea-pigs, this superior sensitivity was not observable, OGI giving equally high agglutination titres.

The results obtained with regard to *enteritidis* "H" antibody are shown in Tables V*a*–V*e* (B and D groups alone) and Tables VI*a*–VI*d* for all four

Table V*a*. *Mean titres for males and females of strains* B *and* D

	Mouse strain			
	B		D	
Sex ...	♂	♀	♂	♀
Mouse titre	3·00	3·93	3·96	4·83
No. of mice	28	27	27	30
Estimated S.E.	0·18	0·28	0·15	0·37

Tube 1 = serum dilution 1 : 25.

Table V*b*. *Difference between the sexes of the same strain*

	Mouse strain	
	B	D
Difference	0·93	0·87
Estimated S.E.	0·33	0·36

Table V*c*. *Difference between* B *and* D *males*

Estimated difference = −0·96
Estimated S.E. = 0·35

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Table V*d*. *Difference between* B *and* D *females*

Estimated difference = −0·90
Estimated S.E. = 0·35

Table V*e*. *Comparison of* B *and* D *regardless of sex*

Estimated difference = −0·93
Estimated S.E. = 0·24

Table VI*a*. *Mean titres for males and females of different strains*

	Mouse strain							
	A		B		D		E	
Sex ...	♂	♀	♂	♀	♂	♀	♂	♀
Mean titre	—	3·50	2·18	2·86	3·50	3·55	2·90	3·20
No. of mice	—	16	28	30	22	20	30	30
Estimated S.E.	—	0·36	0·19	0·22	0·26	0·17	0·26	0·19

The sexual difference is significant only in strain B (difference = 0·688, estimated S.E. = 0·297.)
Tube 1 = serum dilution 1 : 25.

Table VI*b*. *Difference between males of different strains*

	Mouse strains		
	B and D	B and E	D and E
Estimated difference	−1·32	−0·72	0·60
Estimated S.E.	0·34	0·31	0·33

Table VI*c*. *Difference between females of different strains*

	Mouse strains					
	A and B	A and D	A and E	B and D	B and E	D and E
Estimated difference	0·63	−0·05	0·30	−0·68	−0·33	0·35
Estimated S.E.	0·37	0·40	0·37	0·34	0·31	0·34

Table VI*d*. *Comparison of strains regardless of sex*

	Mouse strains					
	A and B	A and D	A and E	B and D	B and E	D and E
Estimated difference	0·78	−0·22	0·26	1·00	−0·52	0·48
Estimated S.E.	0·35	0·36	0·35	0·24	0·22	0·24

For A and D, $t = -0{\cdot}61$; for A and E, $t = 0{\cdot}74$; all others significant.

lines. When the B and D groups were tested alone it was found that females gave a definitely better response in both lines. However, when the experiment was repeated, this difference was only found to be significant in the case of the B group. The B group gives definitely the poorest titres in each experiment. The other three groups form a more or less continuous series. The D group gives the best response, but is not significantly different from the A group, which in its turn is slightly but not significantly superior to the E group. The difference between the D and E groups is twice its standard error and is, therefore, probably significant.

These results are very different from those obtained with *S. typhi murium* "H" antibody. In this case there is no apparent relationship between the ability to produce "H" antibody and the natural resistance of the strains. As will be shown later, the pure lines are not strictly comparable with the A and B strains, but it certainly appears that the B group contains genes making for a higher degree of resistance than the D or E groups, yet it gives significantly lower titres of "H" antibody.

When we turn to the production of "O" antibodies against *S. enteritidis* OGI, no sex differences are to be observed.

Table VII*a*. *Mean titres for males and females of strains* B *and* D

	Mouse strain			
	B		D	
Sex ...	♂	♀	♂	♀
Mean titre	1·29	1·46	2·00	1·96
No. of mice	27	26	27	30
Estimated S.E.	0·12	0·22	0·26	0·29

Tube 1 = serum dilution 1 : 25.

Table VII*b*. *Difference between strains* B *and* D

Estimated difference = −0·61
Estimated S.E. = 0·23

Table VIII*a*. *Mean titres for males and females of different strains*

	Mouse strain							
	A		B		D		E	
Sex ...	♂	♀	♂	♀	♂	♀	♂	♀
Mean titre	—	1·56	1·53	1·83	2·41	2·90	2·13	2·30
No. of mice	—	16	28	30	22	20	30	30
Estimated S.E.	—	0·22	0·18	0·16	0·27	0·23	0·21	0·16

Tube 1 = serum dilution 1 : 25.

Table VIII*b*. *Comparison of strains regardless of sex*

	Mouse strains					
	A and B*	A and D	A and E	B and D	B and E	D and E
Estimated difference	−0·28	−1·25	−0·83	−0·97	−0·55	0·42
Estimated S.E.	0·29	0·31	0·29	0·20	0·19	0·20

* For A and B, $t = -1$ and is not significant.

As far as strain differences are concerned, it will be seen (Tables VII*b* and VIII*b*) that the results are similar to those previously obtained with "O" antibodies for *S. typhi murium* or O 901, except that it appears that the D group is significantly better than the E group, both these groups giving a higher titre than the A and B groups.

Resistance to infection with S. enteritidis *after vaccination*

The animals used in the last three experiments were given test doses of *S. enteritidis*.

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Table IX. *Resistance to test dose of living* S. enteritidis *after inoculation with* enteritidis *vaccine*

Mice			Av. length of life after test dose of 28×10^6 orgs. in days	Av. length of life after test dose of 1400 orgs. in days
Strain	Sex	No.		
A	♂	8	4·7	18·0
	♀	9	4·7	14·0
B	♂	9	2·6	6·7
	♀	10	2·8	6·0
D	♂	11	3·1	7·2
	♀	8	3·1	7·4
E	♂	12	2·9	5·7
	♀	8	2·9	5·9

In the first of these (those tested against "O 901") the mice were divided into two batches, one receiving a large dose, 28 million organisms and the other a small dose, 1400 organisms; in both cases the doses were given intraperitoneally. By this subdivision the statistical significance of the result was diminished, but it can be seen from Table IX that the mean length of life appears to be uninfluenced by sex and that the order of resistance is A > D > B > E. In the A, D and E groups this order is the same as that found previously with unimmunized mice (Schütze *et al.* 1936); however, in the earlier experiment the B and D groups were indistinguishable.

In the second experiment only the B and D mice were available. In this experiment the small dose of 1000 organisms was given *subcutaneously* in order that the rate of killing might be delayed and smaller differences in resistance made observable. As before, no significant effect could be attributed to sex. The B group had a mean length of life of 14·3 days, whilst that of the D group was 19·35 days, the difference is thus 5·06 days and is significant as the probability that this difference is due to chance is only 0·017.

Finally, to test the degree to which immunity had been achieved after inoculation with *S. enteritidis* vaccine, uninoculated controls and the animals used in the last of the three experiments received 4000 living organisms subcutaneously. The full results are shown in Tables X*a* and X*b*. As there were insufficient males of A group mice available for comparative purposes the figures relating to these are omitted. The experiment was terminated on the 95th day after infection and the survivors (of which there were twenty-one altogether) gassed. It is noteworthy that *S. enteritidis* was grown from the spleen of each survivor. For purposes of computation all survivors were classed as having died on the 94th day.

The first point to be dealt with is the effect of sex and it may be said at once that no significant sex difference could be found between inoculated or uninoculated animals.

The next point to be considered is the information concerning inter-line differences amongst the unimmunized groups. The mean lengths of life fall into the following order: A > B > D > E. It will be observed that in the two

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Table X*a*. *Mean expectation of life in inoculated and uninoculated mouse strains*

	A ♀		B ♂		B ♀	
	I	U	I	U	I	U
Mean	53·37	37·53	26·30	19·28	31·67	16·80
Sample size	16	15	27	29	30	30
Estimate of variance of an observation	1269·00	1124·70	840·10	440·60	1217·00	122·00
Estimate of S.E. of mean	8·91	8·66	5·58	3·90	6·37	2·02
Confidence interval of mean	34·39	18·96	14·83	11·29	18·64	12·67
	72·36	56·11	37·77	27·26	44·69	20·93
Percentage survivors	37·50	20·00	12·20	6·80	20·00	0·00

	D ♂		D ♀		E ♂		E ♀	
	I	U	I	U	I	U	I	U
Mean	13·05	10·62	17·63	9·75	9·50	7·72	9·57	8·67
Sample size	22	16	19	20	30	29	30	30
Estimate of variance of an observation	12·33	8·52	358·70	8·51	2·47	2·06	3·69	1·61
Estimate of S.E. of mean	0·75	0·73	4·34	0·65	0·29	0·27	0·35	0·23
Confidence interval of mean	11·49	9·06	8·51	8·39	8·91	7·17	8·85	8·20
	14·61	12·18	26·75	11·11	10·09	8·27	10·29	9·14
Percentage survivors	0·00	0·00	5·30	0·00	0·00	0·00	0·00	0·00

Note. The confidence interval is so determined that the proposition "the true mean value within this interval" has the chance of 95% of being true.

I = immunized. U = unimmunized.

experiments described previously the D group was more resistant than the B group; this reversal is probably due to chance fluctuations in the frequencies of certain genes in the B group. The fact that the lines A and B are genetically heterogeneous can be seen from a comparison of their variances with those of the two pure lines "D" and "E". This difference is reflected in what is known as the confidence intervals of the means. In the case of E group females this interval is 8·20–9·14 days. That is to say, if this experiment were repeated a large number of times under identical conditions the means should fall within these limits in 95% of cases. In the case of B group females the interval is 12·67–20·93 days: in other words, the confidence interval is nine times larger in the case of a genetically heterogeneous strain. Owing to this great difference in variability between the selected strains and the pure lines, it is not permissible to compare their means. If the A and B groups are compared with one another, the mean expectation of life is found not to differ significantly; on the other hand, the D group has a significantly greater expectation of life than the E group.

When the effects of inoculation are studied it will be seen that the variance of the inoculated groups is greater than that of the uninoculated groups in each of the four strains, and it is specially noticeable in the case of the D females. The explanation of this is at present obscure.

In the A group the effect of immunization is not statistically significant. In the B group males there were three survivors (12·2%) as compared with

Table X*b*. *Effect of inoculation on expectation of life in mouse strains*

	A		B		D		E	
	♂	♀	♂	♀	♂	♀	♂	♀
Gain of life	−12·47	15·84	7·02	14·87	2·43	7·88	1·78	0·90
No. of individuals observed	8	31	56	60	38	39	59	60
Estimate of variance	956·20	154·90	45·27	44·63	1·16	18·36	0·15	0·18
Estimate of S.E.	30·92	12·45	6·73	6·68	1·08	4·28	0·39	0·43
Confidence interval	−88·13	−9·62	−6·44	1·51	0·27	−0·68	0·99	0·04
	63·19	41·30	20·48	28·23	4·57	16·44	2·55	1·76
Lower confidence limit of "gain"*	−72·55	−5·51	−4·08	3·85	0·65	0·82	1·14	0·19

* The lower confidence limit has the property that there is 95% chance of truth of the statement that the expected gain of life is above this limit. If this is negative, then the observed gain is insignificant.

two survivors amongst the uninoculated (6·8%). The difference in expectation of life between inoculated and uninoculated is not significant. In the B group females there were six survivors (20%) in the inoculated group and none amongst the uninoculated. The mean gain of life is 14·87 days which is significant. If the survivors are excluded this gain is converted into a loss of 0·72 days with a standard error of 3·74 days. In the D group females there was one mouse among the inoculated whose survival was highly anomalous and inexplicable, the last death previous to this being on the 27th day or 68 days before the end of the experiment. If the survivor is included, the gain is 7·88 days with a standard error of 4·28, whilst if it is excluded the gain is 3·64 days with a standard error of 1·20 days which is highly significant.

It will be noticed that females of the D group appear to have derived greater benefit from inoculation than the males, whilst in the E group the reverse is seen. In neither case is this sex difference significant so that these data give no reason for supposing that one sex is more easily immunized than another. On the other hand, when the lines are compared in this respect, it is seen that the D group, which has the greater natural resistance, is significantly more immunizable than the E group. Thus it appears that for *S. enteritidis* infection, active immunization cannot bridge the gap between strains that differ significantly in their natural resistances.

Discussion

As was stated earlier, one of the prime objects of the present paper was to see whether there was any indication of a correlation between the natural resistance of any strain and its ability to produce antibodies; or, stating the problem in genetic terminology, to see whether there is any reason to suppose that those genes determining a high degree of resistance also tend towards the production of a high titre of antibody.

It will be convenient to consider the "H" antibodies first. So far as the reactions to *S. typhi murium* are concerned, it was found that the more resistant female gave a higher titre than did the male and that the mouse lines fell into the same order when classified according to resistance or to

H" antibody production. Provided that all four groups are really different there are twenty-four different sequences available for the four lines. Therefore, the chance that this particular arrangement should occur fortuitously with both resistance and antibody production is quite small. However, even if the result obtained in the present study is not due to "chance", this must not be taken as proving that the two phenomena are causally related. An example of the fallacies inherent in arguments of this kind may be taken from Bradford Hill's monograph (1934). Hill noticed that the resistance to *S. typhi murium* of two albino strains of mice was greater than that of two coloured strains and concludes that albino mice are more resistant than coloured mice. Actually both the coloured strains were homozygous for the recessive "non-agouti" gene whilst the albino strains carried its dominant allele, the agouti gene. Now Detelfsen & Roberts (1918) showed that the agouti gene conferred a slight advantage over the non-agouti gene in the early weeks of life. It will be seen that the evidence collected by Hill may equally (or perhaps better) be interpreted as indicative of the importance of the agouti gene, of whose existence in the albino strains Hill was obviously unaware. An analogous genetical situation might explain the agreement between resistance and the ability to produce antibodies observed in these experiments.

When we turn to a consideration of *S. enteritidis* "H" antibody production and resistance, no evidence of any correlation can be shown. Actually in the last experiment an attempt was made to detect a mathematical correlation between the mean titres and expectations of life; however, the value of the correlation coefficient was found to be less than 0·5.

In the case of "O" antibody production it was found that the two pure lines (D and E) gave higher titres with all organisms (*S. typhi murium*, *S. typhi* "O 901" and *S. enteritidis* "OGI", than did the two selected strains (A and B). With the normal antibody to "O 901" there was some evidence of a negative interlinear correlation between the mean titre and resistance. In the case of antibody to *S. typhi murium* positive correlation was established only so far as sex is concerned. In other cases there was no evidence of any kind of correlation. As has been pointed out before, it is doubtful whether there is any statistical justification for comparing the mean lengths of life of the pure lines and the selected strains. However, it is justifiable to deduce in the last experiment with *enteritidis* immunization and infection, that the A and B strains possess genes making for a higher degree of resistance than any present in the D and E strains. If these genes had a marked influence on antibody production we should expect the relative titres to be the reverse of those found. If there were a negative relationship between resistance and "O" antibody production we should expect the E group to give a better response than the D group, when in fact the reverse was found.

It must not be thought that the above paragraphs are intended to invalidate the statements that have been written concerning the relative importance of the "H" and "O" *antigens* in immunization against *Salmonella*

infections. The evidence in the latter case is of quite a different kind and, indeed, both Topley (1929) and Schütze (1930) agree that a low titre of antibodies is consistent with a high degree of immunity.

It is perhaps noteworthy that although inbreeding appears to make a great deal of difference to variability in the expectation of life following infection (Table X*a*), as shown by a comparison of the pure lines and selected strains, this difference in variance is not noticeable so far as antibody production is concerned, as can be seen from a comparison of the standard errors shown in the tables. In other words, the amount of circulating antibody appears to be far less under genetic control than is the case with the survival time following infection. It would be interesting to see whether there was any correlation between survival time and titre in individuals drawn from the same pure line.

Apart from the problems dealt with above, the experiment illustrated in Tables X*a* and X*b* showed the potential value of pure lines in bacteriological research and biological standardization. Dr Hsu has calculated the number of mice in the B and E strains that would have to be used in order to have a 50% chance of detecting a gain in the expectation of life following immunization (using the data for males in Table X*a*). If the "real" gain of life were one day, about 3360 B males would be needed, whilst about 30 E mice would give the same result. The number of E mice needed is approximately one-hundredth of the number of B mice. For practical purposes it would be necessary to have more than a 50% chance of detecting the difference but the relative values of the two strains are well shown in the figures given. Similarly, in the detection of the difference in immunizability between strains of different resistance, it might be thought preferable to use the widely contrasted A and E strains. Although the A females have an observed gain of 15·84 days, this figure is not significant, whilst a gain of 0·9 day for the E group females is significant. No significant information can be obtained from a comparison of these two strains.

There have been comparatively few experiments relating to the effect of inbreeding on response to infection. Irwin (1933) used *S. enteritidis* in inbred strains of rats and emphasized that the uniformity of response could be improved by selection even in strains of rats that had been brother-sister inbred for over forty generations. Haldane (1936) has dealt with the occurrence of the heterogeneity in stocks that have been brother-sister inbred for a number of generations and it is frequently advisable that inbreeding should be supplemented by selection for the greatest degree of uniformity. Hezler (1937) has shown that inbreeding of selected resistant strains of mice increases their homogeneity without diminishing their degree of resistance to *S. typhi murium*.

The great difference in variability observed in this series of experiments between the selected strains and pure lines is somewhat surprising. The best way to show the magnitude of genetic variability is a comparison of the variances of two pure lines, their F_1 hybrids and the F_2 hybrids (the progeny of the F_1). It is worth emphasizing the fact that the F_1 hybrids of two pure

lines should be as homogeneous as the pure lines themselves. Mendelian segregation occurs in F_2 and should result in a great increase in variance. It is only fair to Webster to state that his susceptible strain has been considerably more inbred than the B strain. Further, his mode of infection (*per os*) is possibly a more delicate method of administering the organisms than that used here. On the other hand, his method of assessing results, viz. in terms of per cent mortality, is cruder than that used here and would obscure a great deal of the internal variation of his strains. To obtain uniformity of response, inbreeding alone is considerably more effective than selection alone; the optimum results would doubtless be obtained by a combination of inbreeding and selection.

SUMMARY

1. Four mouse lines have been tested for "H" and "O" antibody production following inoculation with *Salmonella typhi murium* and *S. enteritidis*; two of these lines had been selected for resistance and susceptibility but not inbred; two, D and E, had been brother-sister inbred for over thirty generations but not selectively as far as resistance is concerned.

2. After immunization with *S. typhi murium*:

(*a*) Female mice, in all four lines, tend to give higher "H" and "O" titres than do males, a positive correlation between ability to produce antibody and resistance to infection being thus established.

(*b*) Interline differences exist for both these antibodies. Those concerned with "H" antibody may be correlated with resistance. In the case of "O" antibody, no correlation is suggested.

3. After immunization with *S. enteritidis*:

There appears to be no correlation sexually or interlineally between resistance and antibody production. That genetic differences exist, in respect of this organism also, is shown by the fact that the pure lines, D and E, give a better antibody response than the selected lines A and B. When tested for immunizability by subsequent infection with *S. enteritidis* no useful information could be obtained with strain A or B owing to their great internal variation. Strains D and E both showed a gain in expectation of life following immunization. The more resistant strain D showed a significantly greater gain than did E, the strain with a lower natural resistance.

4. The titres of normal "O" *enteritidis*-antibody found in the four lines indicates that there may be a negative correlation between the titre of these antibodies and resistance to infection with *S. enteritidis*.

5. The significance of the above findings is discussed and it is stressed that a significant correlation between resistance and antibody formation does not imply that the two phenomena are causally related.

6. It is pointed out that strains that have been inbred even without selection react far more homogeneously in the infection experiments here described than do strains that have been selected but not intensively inbred.

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REFERENCES

DETELFSEN, J. A. & ROBERTS, E. (1918). *Genetics*, **3**, 573.
FELIX, A. (1930). *Lancet*, **1**, 505.
GADDUM, J. H. (1933). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 183.
GOWEN, J. W. (1936). *Genetics*, **21**, 1.
HALDANE, J. B. S. (1936). *J. Genet.* **32**, 315.
HETZLER H. O. (1937). *Genetics*, **22**, 264.
HILL, A. BRADFORD (1934). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 196.
IRWIN, M. R. (1933). *J. Immunol.* **24**, 285.
MACMASTER, P. D. & HUDACK, S. S. (1934). *Proc. Soc. exp. Biol., N.Y.*, **31**, 751.
NEYMAN, J. (1937). *Philos. Trans.* A, **236**, 338.
NEYMAN, J. & JOHNSON, O. P. (1936). *Statist. Res. Mem.* **1**, 57.
SCHÜTZE, H. (1930). *Brit. J. exp. Path.* **11**, 34.
SCHÜTZE, H., GORER, P. A. & FINLAYSON, M. (1936). *J. Hyg., Camb.*, **36**, 37.
TOPLEY, W. W. C. (1929). *Lancet*, **1**, 1337.
WEBSTER, L. T. (1933). *J. exp. Med.* **57**, 793.
—— (1937). *J. exp. Med.* **65**, 261.

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