IMMUNISATION AND SELECTION AS FACTORS IN HERD-RESISTANCE[1]

By W. W. C. TOPLEY, J. WILSON and E. R. LEWIS.

(*From the Department of Bacteriology and Preventive Medicine, University of Manchester.*)

In considering the factors which are concerned in the spread of any epidemic bacterial infection among any host-population, the resistance brought into play by the latter will clearly be of crucial importance.

It has been suggested (Topley and Wilson, 1923) that the general problems of immunity and resistance have been studied too exclusively from the point of view of the individual, and that their reconsideration from the point of view of the herd would probably yield results of some interest. It is clear, for instance, that the herd possesses at least one method of acquiring resistance that is not at the disposal of the individual. If natural resistance be in any degree a variable character, the average herd resistance will be augmented by a simple sorting process during the spread of any epidemic infection associated with specific deaths among the population at risk, quite apart from any possible increase in the resistance of surviving individuals within that population.

As regards the spread of enteric infection among mice, we have learned that the survivors left after the passage of one epidemic wave show relatively high resistance, when exposed along with fresh susceptibles, to the risk of a second outbreak of the same disease. The level of resistance reached, however, affords no complete protection against a subsequent fatal infection. Although the survivors from the first wave tend to escape death during the early phases of the second epidemic, many of them succumb during its later stages (Topley, 1921; Amoss, 1922). Have these survivors acquired resistance during their exposure to the risk of infection, or do they merely represent that quota of the original population whose resistance, from the start, has been at a high level?

That variations in resistance to any given bacterial parasite do in fact occur among mice, as among all other animal species, has long been accepted, partly on the ground of actual observation, and largely on the ground of *a priori* probability. We owe to Webster the clear demonstration that, in the case of mouse typhoid, such variations are significant in degree.

Natural selection, then, will be provided with material susceptible to its inherent sorting action. It will certainly be operative during any one epidemic of disease, and will certainly tend to raise the average resistance to that

[1] A report to the Medical Research Council.

https://doi.org/10.1017/S002217240003429X Published online by Cambridge University Press

disease of the population at risk, provided that fresh susceptibles do not gain admittance during the epidemic period. Can we decide with any certainty whether or no, in the particular case of mouse typhoid, the acquirement of increased resistance by individual mice plays an additional part?

Data already available.

Some light on this question may be gained by a study of the attempts which have been made to immunise mice against organisms of the enteric group, and especially against *B. aertrycke* (mutton), which is the chief cause of enteric infection among these animals. If active immunisation of the mouse against *B. aertrycke* were an easy procedure, attended with but slight mortality arising directly from the administration of the bacterial antigen, and resulting in a high degree of resistance, it would be reasonable to attribute to the development of an acquired immunity, during the actual spread of infection, an important share in bringing the epidemic to a close. If, on the other hand, it be found that such immunisation is difficult to carry out, often leading by itself to a high mortality among the treated mice, and that, even so, the resistance acquired is uncertain in its incidence and relatively slight in degree, then we should hesitate to believe an acquired immunity of this kind to be an important factor in checking the natural spread of disease.

It may be stated at once that, while certain of the earlier attempts at active immunisation against mouse typhoid yielded some degree of success (Loeffler, 1906; Wolf, 1908; Yoshida, 1909), the preliminary treatment was in most cases so severe as to result in a very considerable mortality, in some cases amounting to 50 per cent. of the treated animals. Within the last few years a growing realisation of the importance of studying the response of laboratory animals to those infections to which they are naturally liable, rather than their reaction to the injection, directly into their tissues, of bacteria which, so far as we know, do not attack them under natural conditions, has led to a somewhat extensive study of mouse typhoid in this country, in America and in Germany.

In Tables I to V[1] are summarised such of the experimental data available as bear directly on the question at issue. Many of these figures have been collected, in a somewhat similar form, by Neufeld (1924) whose general discussion deals with many of the fundamental problems of active immunisation.

It is not, we think, necessary to deal at any length with the data tabulated. It is clear that some degree of increased resistance against intraperitoneal or subcutaneous infection can be produced by parenteral administration of killed bacteria, in doses not of themselves lethal (Table I), and that this resistance is increased by increasing the number of preparatory inoculations (Webster, *i*, *k* and *m*). This increase in resistance is, however, relatively slight in degree, and is evidenced rather by a lengthening of the average time to death after

[1] The doses given in these tables have in all cases been calculated, as nearly as the protocols allow, in terms of the number of bacteria injected.

Table I.

Immunisation against B. aertrycke *with dead cultures administered subcutaneously. Tested by intraperitoneal or subcutaneous inoculation.*

	Method of immunisation					Method of testing immunity							
	Living or dead bacteria	Route of administration	Dose in bacilli	No. of times administered	Mortality % from immunisation	Route of administration	Dose in bacilli	No. of mice tested	Mortality % in test	No. of control mice in test	Mortality % in controls	Mortality ratio. Controls: immunised	Av tin de in imn
vi. 83	D.	Sc.	$2{\cdot}5\times10^{8}$	1	0	Ip.	5×10^{6}	10	100	2	100	1	
	D.	Sc.	$2{\cdot}5\times10^{8}$	2	0?	Ip.	5×10^{6}	20	90	3	100	1·1	1(
	D.	Sc.	$2{\cdot}5\times10^{8}$	3	0?	Ip.	5×10^{6}	20	70	3	100	1·4	1
2	D.	Sc.	2×10^{8}	1	0	Sc.	10^{6} to 10^{10}	11	100	5	100	1	
	D.	Sc.	10^{9} and 2×10^{9}	2	0	Sc.	10^{4} to 10^{10}	10	90	11	100	1·1	
f)	D.	Sc.	$1{\cdot}3\times10^{10}$ in all	8	0	Sc.	10^{2}	3	100	2	100	1	
h)	D.	Sc.	$1{\cdot}75\times10^{10}$ in all	8	33	Sc.	10^{2}	2	0	?	100	∞	

https://doi.org/10.1017/S002217240003429X Published online by Cambridge University Press

the test inoculation, than by any marked increase in the proportion of ultimate survivors.

It appears (Table II) that inoculation of such killed bacterial antigens yields some definite protection against infection per os, as tested by intra-stomachal injection (Webster, *j*, *l* and *n*), feeding with a dropping pipette (Lange and Yoshioka, *g*, *j*, *k* and *l*), feeding on bread soaked in culture (Topley and Wilson, *a*) or simple exposure to the risk of infection from other infected mice (Topley and Wilson, *b*). In the experiments recorded in this table there is not the same lengthening of the average time to death when the immune mice are compared with the controls, as was observable when the test dose was administered intraperitoneally or subcutaneously. It appears, in both tables, that the results obtained in this country and in America have yielded a far higher proportion of successes, as regards immunisation, than have been met with by Neufeld and his collaborators. This may, in part, be due to a difference in the pathogenicity of the strains of *B. aertrycke* employed. It may, in part, be due to the fact that, since relatively smaller numbers of mice were employed by the German workers, it was necessary to ensure the death of the controls, rather than rely on the comparative mortalities in the test and control groups, and hence relatively large test doses of living bacteria had to be employed.

Table IV shows that oral administration of living bacteria may lead to the acquirement of a relatively high degree of immunity to subsequent inoculations of living bacteria into the tissues, and that there is some association between a high mortality during the period of immunisation and the degree of resistance ultimately obtained (compare Webster, *c*, *f* and *h* with each other and with Lange and Yoshioka, *e*).

Table V shows that the same method of treatment results in a definite increase in resistance to subsequent administration of living *B. aertrycke* per os. The disparity between the results obtained at the Rockefeller Institute (Webster, *d*, *e* and *g*) and those reported from the Robert Koch Institute (Lange and Yoshioka, *a*, *b*, *c* and *d*) are here very marked.

Experiment.

In connection with another experiment, not yet reported, we desired to produce faecal excretion of *B. aertrycke* in a large number of mice. For this purpose 185 mice were fed, on several occasions during a period of one month, with broth cultures of this organism. At each feeding 0·02 c.c. of a 1/10 dilution of an 18 hours' broth culture, grown at 22° C., was administered per os to each mouse with a graduated dropping pipette.

One month after the last feeding the position was as follows. Of the 185 mice, 92 had died, in most cases from typical *B. aertrycke* infection. Of the remaining 93, 61 had excreted *B. aertrycke* on one or more occasions. The remaining 32 mice had never yielded cultures of this organism from the faeces, though specimens from each mouse had been repeatedly examined.

Table II.

ınisation against B. aertrycke *with dead cultures administered subcutaneously or intraperitoneall*
Tested by administration per os or by exposure to risk of infection.

	Method of immunisation					Method of testing immunity							Ave tin de in d imm
	Living or dead bacteria	Route of adminis-tration	Dose in bacilli*	No. of times adminis-tered	Mortality % from immuni-sation	Route of adminis-tration	Dose in bacilli	No. of mice tested	Mortality % in test	No. of control mice in test	Mortality % in controls	Mortality ratio. Controls: immunised	
XVI. 83	D.	Sc.	$2{\cdot}5\times10^{8}$	1	0	P.O.	5×10^{6}	10	100	2	100	1	1
	D.	Sc.	$2{\cdot}5\times10^{8}$	2	0?	P.O.	5×10^{6}	20	55	3	100	1·8	2
	D.	Sc.	$2{\cdot}5\times10^{8}$	3	0?	P.O.	5×10^{6}	20	40	3	66·7	1·7	1
) 1	D.	Sc.	$1{\cdot}3\times10^{10}$ in all	8	0	P.O.	10^{10}	3	100	—	—	—	
)	D.	Sc.	$1{\cdot}75\times10^{10}$	8	33·3	P.O.	10^{10}	2	50	?	100	2	
)	D.	Sc.	$2{\cdot}5\times10^{10}$ in all	11	82	P.O.	10^{10}	2	50	5	80	1·6	
)	D.	Sc.	$2{\cdot}7\times10^{10}$ in all	6	66·7	P.O.	10^{10}	4	100	—	—	—	
. 476	D.	Sc.	?	4	40	P.O.	10^{10}	6	100	1	100	—	
	D.	Ip.	5×10^{8} and 10^{9}	2	26	F. 3 times and E.	?	90	54·4	90	88·9	1·6	
	D.	Ip.	5×10^{8} and 10^{9}	2	31·8	E.	?	75	8	75	41·3	5·2	

*=In this and all tables the dose is estimated as closely as possible from the information given in the protocols.
P.O.=Administration per os by a dropping pipette or a catheter.
F.=Feeding on bread soaked in a culture of *B. aertrycke*.
E.=Exposure to risk of infection.

https://doi.org/10.1017/S002217240003429X Published online by Cambridge University Press

Table III.

Immunisation against B. aertrycke *with dead cultures administered per os. Tested by intraperitoneal inoculation or per os.*

Method of immunisation					Method of testing immunity							
Living or dead bacteria	Route of administration	Dose in bacilli	No. of times administered	Mortality % from immunisation	Route of administration	Dose in bacilli	No. of mice tested	Mortality % in test	No. of control mice in test	Mortality % in controls	Mortality ratio. Controls: immunised	Average time to death in days: immunised
D.	F.	?	28	0	P.O.	10^7	17	58·8	4	75	1·2	18·1
D.	F.	?	28	0	Ip.	10^7	16	75	4	100	1·3	6·6
D.	P.O.	?	4	40	P.O.	10^{10}	18	83·3	1	100	—	—

F. = Fed on bread soaked in cultures of *B. aertrycke*.
P.O. = Administered per os with a dropping pipette or with a catheter.

Table IV.

Immunisation against B. aertrycke *with living cultures administered per os. Tested by intraperitoneal or subcutaneous inoculation.*

Method of immunisation					Methods of testing immunity							
Living or dead bacteria	Route of administration	Dose in bacilli	No. of times administered	Mortality % from immunisation	Route of administration	Dose in bacilli	No. of mice tested	Mortality % in test	No. of control mice in test	Mortality % in controls	Mortality ratio. Controls: immunised	Average time to death in days: immunised
L.	F.	?	5 to 13	28	Ip.	10^6	18	22·2	7	100	4·5	9
L.*	F.+P.O.	$?+2\times10^6$	30†+ 1 P.O.	54	Ip.	2×10^6	11	0	5	100	∞	—
L.	F.+P.O.	$?+10^7$	34†+ 1 P.O.	66·7	Ip.	2×10^6	6	0	5	100	∞	—
L.	P.O.	10^5 to 10^9	1	0	Sc.	10^4	6	100	2	100	1	8·3

=Feeding on bread soaked in a culture of *B. aertrycke*.
=Administration per os by a dropping pipette or a catheter.
=These mice were fed on an incompletely sterilised vaccine.
=Number of days over which feeding was continued. This was followed by one administration of living culture per os.

https://doi.org/10.1017/S002217240003429X Published online by Cambridge University Press

Table V.

Immunisation against B. aertrycke *with living cultures administered per os. Tested per os.*

	Method of immunisation					Method of testing immunity							
	Living or dead bacteria	Route of administration	Dose in bacilli	No. of times administered	Mortality % from immunisation	Route of administration	Dose in bacilli	No. of mice tested	Mortality % in test	No. of control mice in test	Mortality % in controls	Mortality ratio. Controls: immunised	A t i
[xix. 130	L.	P.O.	$4{\cdot}5\times10^{6}$	1	30	P.O.	4×10^{6}	54	29·6	20	80	2·7	
	L.?*	F.	?	30†	50	P.O.	10^{7}	12	8·3	12	58·3	7	
	L.	F.	?	34†	61·1	P.O.	10^{7}	7	14·3‡	7	100	7	
(*a*) [57	L.	P.O.	10^{5} to 10^{7}	1	0	P.O.	10^{9}	9	44·4	6	33·3	0·75	
(*b*)	L.	P.O.	10^{5} to 10^{9}	1	0	P.O.	10^{9}	7	85·7	6	83·3	0·97	
(*c*)	L.	P.O.	10^{5} to 10^{7} $+10^{9}$	2	40	P.O.	10^{9}	6	83·3	6	83·3	1	
(*d*)	L.	P.O.	10^{7}	1	33·3	P.O.	10^{9}	6	66·7	4	100	1·5	

*=These mice were fed on an incompletely sterilised vaccine.
†=Number of days over which feeding was continued.
‡=The mouse which died showed no definite evidence of *B. aertrycke* infection.
F.=Fed on bread soaked in cultures of *B. aertrycke*.
P.O.=Administered per os with a dropping pipette or with a catheter.

https://doi.org/10.1017/S002217240003429X Published online by Cambridge University Press

These 32 mice, therefore, presented a quota of the original population which had been selected in two ways. They formed a sample of a surviving group which amounted in all to half the original population. They formed a unique group, representing 17·3 per cent. of the original population, characterised by the fact that they had survived for one month or more since the last feeding, without ever having been known to excrete *B. aertrycke* in their faeces.

A specimen of blood was obtained from each mouse and tested against type and group suspensions of *B. aertrycke* in a series of dilutions commencing at 1/20. The serum from one mouse gave a trace of agglutination at a dilution of 1/20 with the type suspension, and standard agglutination at 1/160 with the group suspension. The sera from the other 31 mice gave completely negative results.

We decided to test these mice for resistance, by intraperitoneal inoculation, in order to discover whether their apparent escape from infection was associated with an increased resistance to *B. aertrycke* when this organism was introduced directly into the tissues. The first two horizontal lines of Table VI show the result of injecting each of the 32 mice, together with 30 controls, with 200,000 *B. aertrycke* administered intraperitoneally. All mice which died were submitted to post-mortem examination according to the technique which has been recorded in many previous reports.

The results need little comment. During the 21 days which followed the intraperitoneal injection, the total and specific mortality among the controls was 76·7 per cent. Among the selected mice the total mortality was 21·9 per cent., the specific mortality was 12·5 per cent. The average time to death among the controls which succumbed to infection was 7·4 days; among the selected mice which died of *B. aertrycke* infection it was 5 days. The selected mice were apparently about six times as resistant as a normal sample of mice, judged from the specific mortality. They represented a selected sample of about one-sixth of the normal population from which they were originally derived, though their selection on a basis of survival was only in the proportion of one-half.

These selected mice were clearly exceptionally resistant to *B. aertrycke*, when introduced into their tissues, and the results, at this stage, were entirely compatible with the view that their escape from infection during the early stages of the experiment was the result of a natural resistance, which had remained unaltered during this period.

As the result of this first intraperitoneal injection, followed by observation over 21 days, the sample of 32 mice had been subjected to a further sorting process, leaving 25 mice, which formed a sample amounting to 13·5 per cent. of the original 185 mice.

These 25 mice, together with 25 controls, were now injected intraperitoneally with 20,000,000 *B. aertrycke*, and were, thereafter, observed for 68 days. The results are shown in Series C and D of Table VI. All the controls

died within 11 days and all from typical *B. aertrycke* infection. The average time to death was 5·2 days. By the time the last control had died three of the 25 selected mice had succumbed to the same infection. By the 68th day the total mortality among the selected mice was 44 per cent., and the specific mortality 28 per cent. The average time to death among those selected mice which died from *B. aertrycke* infection was 25·6 days. More than half of these selected mice, therefore, withstood for 68 days an infection which killed all the individuals of an equal sample of normal mice within 11 days. Clearly, the previous intraperitoneal inoculation had not acted as a simple selective agent. The mice which survived its effects for 21 days were possessed of a degree of resistance to *B. aertrycke*, when introduced into the tissues, not found among a normal sample. There had been immunisation as well as selection.

Table VI.

Showing results of successive intraperitoneal inoculations carried out on a selected sample of mice.

Series	Nature of mice	No. of mice	Bacteria inoculated	Dose administered in bacilli	Days observed	Days to death	Average time to death in days: specific deaths	Total mortality %	Specific mortality %
A	Surviving non-excretors after feeding (see text)	32	*B. aertrycke*	2×10^5	21	3, 4, 6, 7, 8*, 16*, 18*	5	21·9	12·5
B	Normal controls to A	30	*B. aertrycke*	2×10^5	21	1, 2, 2, 3, 3, 3, 4, 4, 5, 6, 6, 8, 8, 8, 9, 9, 10, 11, 11, 11, 13, 15, 18	7·4	76·7	76·7
C	Survivors from A	25	*B. aertrycke*	2×10^7	68	5, 8, 9, 15, 31*, 34, 40, 50*, 54*, 63*, 68	25·6	44	28
D	Normal controls to C	25	*B. aertrycke*	2×10^7	68	2, 2, 3, 3, 3, 3, 3, 3, 3, 4, 4, 4, 4, 4, 5, 6, 6, 6, 7, 8, 8, 9, 9, 11, 11	5·2	100	100
E	Survivors from C	14	*Pasteurella*	5×10^2	28	1, 1, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2	1·8	92·9†	92·9
F	Normal controls to E	20	*Pasteurella*	5×10^2	28	2, 2, 2, 2, 2, 2, 2, 2, 2, 3, 3, 3, 3, 3, 3, 3, 3, 4, 4	2·65	100	100
G	Normal controls to E	3	*Pasteurella*	5×10	28	2, 3, 3	2·7	100	100
H	Normal controls to E	3	*Pasteurella*	5	28	2	2	33·3	33·3

*=Death not due to *B. aertrycke* infection.
†=The surviving mouse from this group was re-tested ten days later and found susceptible (see text).

In the studies referred to above, Webster has shown that, among any considerable sample of mice from an inbred strain, a certain proportion will survive infection, and a proportion of these survivors will act as though they were entirely refractory, in the sense that they will neither yield cultures of *B. aertrycke* from their faeces, nor from their blood, nor is it possible to demonstrate agglutinins for that organism in their serum. Using a single strain of *B. aertrycke* this proportion is constant, within moderately narrow limits, for different samples of the same inbred strain, but varies with different strains of mice. Using different strains of *B. aertrycke*, that is, strains which

would be placed in this group on general cultural and serological grounds, but which have been derived from different sources, and have long been held in stock in different laboratories, and keeping the host factor constant by using a single strain of inbred mice, Webster showed that the result of administration per os differed with the different bacterial strains, as regards the percentage of surviving and refractory mice. These differences became more marked when comparison was made between the action of different bacterial species within the *B. paratyphosus* B–*B. enteritidis* group (Webster, 1923, *a*, *b*, *c* and *d*).

It seemed probable that the host-resistance displayed in such experiments would prove to be non-specific in character, and Webster proceeded to test this point by submitting mice to the action of a metallic poison, mercuric chloride. He showed (Webster, 1923 *d*) that by administering suitably graded doses of this substance per os to samples of mice, mortality curves could be obtained similar in their general form to the curves obtained after infection with *B. aertrycke*. In later series of experiments he showed that mice, which had survived one or more doses of mouse typhoid bacilli, were more resistant than normal mice to the action of mercuric chloride (Webster, 1924 *a*), that, when a strain of mice unusually resistant to *B. aertrycke* infection has been produced by selective breeding, such mice are also unusually resistant to mercuric chloride (Webster, 1924 *b*), and that mice, whose resistance to *B. aertrycke* infection has been raised by a particular diet, show an increased resistance to this chemical poison. In this last series of experiments it was also shown that the resistant mice were less susceptible than normal mice to the intraperitoneal inoculation of Botulinus toxin (Webster and Pritchett, 1924).

In another series of experiments (Webster, 1924 *a*), it was demonstrated that the survivors from various groups of mice, to which had been administered different strains of the paratyphoid-enteritidis group of bacteria, showed in all cases an increased resistance to *B. aertrycke* infection, and that the increase in resistance seemed to depend rather on the virulence of the strain used in the primary infection, than on the closeness of the antigenic relationship between the primary infecting strain and *B. aertrycke*.

In the experimnet at present under discussion, we had found that a group of mice, selected on the grounds of their apparent failure to react to the ingestion of *B. aertrycke*, were unusually resistant to the intraperitoneal inoculation of this organism, and that, three weeks after this intraperitoneal inoculation, they showed a degree of resistance without parallel among a sample of normal mice. It appeared of interest to test whether these mice were possessed of any increased resistance to an entirely unrelated bacterial infection.

For this purpose we took the 14 survivors from Series C (Table VI), and inoculated them intraperitoneally with 500 *Pasteurella muris*. At the same time we inoculated twenty controls with the same dose, three controls with 50,

and three with 5 *Pasteurella*. We purposely employed a relatively large dose of *Pasteurella*, because the test was, from its nature, one which could not be repeated, and it was necessary to be certain of a high mortality among the controls. In our hands the minimal lethal dose of *Pasteurella* has varied widely, from a single organism to many thousands, when injections are made intraperitoneally into mice.

The results are shown in Series E, F, G and H (Table VI). Of the 14 survivors, 13 died within 2 days. Of the twenty controls which received the same dose, all died within 4 days. Of the three controls which received 1/10th of this dose, all died within 3 days. Of the three controls which received 1/100th of this dose, one died in 2 days and two survived without any evidence of illness. Thus, the 14 survivors from the *B. aertrycke* infections received between 10 and 100 minimal lethal doses of *Pasteurella*. The 13 mice which died showed no delay in the fatal issue. Their average time to death was 1·8 days. The average time to death of the twenty controls which received the same dose was 2·65 days.

The single selected mouse which survived the injection of *Pasteurella* showed no ill-effects whatever. Since it seemed unlikely that one mouse would prove entirely refractory, while its 13 companions seemed more susceptible than the normal controls, the suspicion arose that some error had occurred in the inoculation, and that this mouse had not received the full dose intraperitoneally. After an interval of some 10 days, therefore, this mouse was re-inoculated with the same dose, together with two controls. The selected mouse died in 24 hours, the two controls on the second day.

It would appear that a group of mice, selected by survival after repeated infections with *B. aertrycke* and possessing an enormously increased resistance to that organism, show no increased resistance to *Pasteurella muris*, when compared with a sample of normal mice.

LATENT *B. AERTRYCKE* INFECTION AMONG THE SELECTED MICE.

The 14 surviving mice from Series C had been observed for 68 days following the second intraperitoneal injection of *B. aertrycke*. At autopsy, carried out after their death from acute *Pasteurella* infection, a portion of the spleen from each mouse was transferred to a tube of broth and incubated for 24 hours. A copious growth of *Pasteurella* was obtained in each case. From these broth cultures plates of McConkey's lactose-bile-salt medium were inoculated, and any non-lactose fermenting colonies were subcultured and tested by agglutination.

From the spleens of 8 of the 14 mice, cultures of *B. aertrycke* were obtained in this way. It is possible that the abundant growth of *Pasteurella* in the broth cultures hindered the development of *B. aertrycke*, and that, in the absence of this disturbing factor, the proportion of positive results would have been higher. In any case it is demonstrated that the majority of the selected groups were still harbouring *B. aertrycke* in their tissues.

THE PRESENCE OF AGGLUTININS IN THE BLOOD OF INFECTED MICE.

The rôle of humoral immunity as a factor in survival has been briefly discussed in a recent report (Topley, Ayrton and Lewis, 1924) on the basis of the evidence afforded by the demonstration of agglutinins in the blood of surviving mice.

The relevant data elicited in the course of the present experiment are set out in Table VII.

Table VII.

Showing results of agglutination tests in the selected group of mice at various dates.

Record number of mouse*	Time of test: Before first intraperitoneal inoculation — Type	Before first intraperitoneal inoculation — Group	14 days after first intraperitoneal inoculation — Type	14 days after first intraperitoneal inoculation — Group	63 days after second intraperitoneal inoculation — Type	63 days after second intraperitoneal inoculation — Group
No. 47	Trace 20	160	80	320	D.	D.
,, 49	—	—	—	640	D.	D.
,, 56	—	—	—	40	D.	D.
,, 57	—	—	—	40	Trace 20	640
,, 63	—	—	—	40	Trace 20	80
,, 27	—	—	—	—	Trace 20	40
,, 43	—	—	—	—	640	640
,, 60	—	—	—	—	320	—
,, 62	—	—	—	—	—	160
,, 67	—	—	—	—	Trace 20	—
,, 80	—	—	—	—	—	80
,, 84	—	—	—	—	20	80
No. of mice tested	32		27		16	
No. of mice +	1		5		9	
Percentage of mice +	3·1		18·5		56·25	

* = This number refers to the record number of the mouse in the original population and has no other significance. Mice which acted negatively throughout are not recorded individually, but are included in the number of mice tested.
D. = Died before this date.

The following facts are clearly shown. Although the selected groups of 32 mice when first tested by intraperitoneal inoculation were about six times as resistant as a sample of normal mice, only one of the 32 mice showed the presence of agglutinins in its serum. Clearly, the presence of agglutinins is no measure of resistance, a fact frequently demonstrated in previous experiments of our own, as also by Webster and by many other workers.

After each intraperitoneal inoculation the percentage of mice showing the presence of agglutinins markedly increases, so that 14 days after the first inoculation 18·5 per cent. of the mice reacted positively, and 63 days after the second inoculation this proportion had been increased to 56·25 per cent. Mice surviving the first intraperitoneal inoculation had acquired a definite increase of resistance as the result of their experience. The mice surviving the second intraperitoneal inoculation were not tested as regards their resistance to *B. aertrycke*, so that we cannot include them in the argument.

It seems clear, however, that if we select mice by submitting them to the action of successive and increasing doses of living *B. aertrycke*, we shall find that increasing resistance is associated with an increasing proportion of positively reacting mice as judged by agglutination.

Only one series of tests in this experiment enables us to make a direct comparison between the fate of mice with agglutinins and mice without, where both groups have passed through the same experience, and both are submitted to the same test inoculation. Unfortunately the figures are very small. On the day of the second series of intraperitoneal tests, the sera of four of the 25 selected mice agglutinated *B. aertrycke* at a dilution of 1/20 or over. The sera of the remaining 21 mice failed to react at this dilution. Of the four agglutinators two died during the next 68 days. One showed the typical lesions of *B. aertrycke* infection, the other showed no such lesions, but *B. aertrycke* was isolated by direct plating from its spleen. Of the 21 non-agglutinators, nine died during this period, six of *B. aertrycke* infection and three from other causes.

There is, then, no evidence that the mice with agglutinins were more resistant than their companions, but the numbers involved are too small to have any real significance.

DISCUSSION.

The indications afforded by the experiments recorded in this report, when considered in the light of the results obtained by other workers, appear to be of some interest.

Reviewing the whole of the data together, it is clear that some increase in resistance to *B. aertrycke* may be obtained by the administration of killed cultures of this organism. The level of resistance attained appears never to be very high, and, where the test dose is given parenterally, it is exhibited rather by a delay in the time to death than by an increase in the percentage of survivors, when the mice are observed over a considerable period. When the test dose is given per os, or the treated mice are exposed to the risk of infection, the resistance, as judged by survival, appears to be more effective. The parenteral administration of killed cultures appears to be far more effective in increasing resistance than is their administration per os.

The most striking examples of increased resistance to *B. aertrycke* are, however, to be found among those mice which have survived the administration of living cultures of this organism, whatever route is employed for the test dose. In almost all such cases a certain mortality occurs, following the immunising dose, so that the surviving mice form a selected group, altogether apart from any increase in resistance which may have occurred as the result of the procedure employed for immunisation. There is, moreover, a general parallelism between the severity of the selective process and the degree of resistance exhibited by the survivors, as judged by the percentage mortality and the average time to death following a subsequent dose of living organisms.

Is the increased resistance of these surviving groups the result of simple selection, or is it the result of some change in the condition of the individual hosts brought about by the previous treatment? It appears to us that there is little doubt that such a change is involved, and is a factor of crucial importance.

We have, in many previous reports, commented on the high proportion of latent infections found among mice which have survived the administration or ingestion of *B. aertrycke*. In a recent paper (Topley and Ayrton, 1924) we tabulated the results of a series of experiments bearing on this point, and we can now add to the data there given further figures, obtained in experiments not yet recorded. The figures at present available are summarised in Table VIII.

Table VIII.

Showing the proportion of positive spleen cultures obtained P.M. from 431 *mice which had survived the administration of* B. aertrycke, *or exposure to risk of infection.*

Group	History of Group	Number of mice in group	Number of mice with positive spleen cultures	Percentage of mice with positive spleen cultures
1	Survived for 21 days after intraperitoneal or subcutaneous injection of *B. aertrycke*	50	44	88
2	Survived for 42 days after one feeding with 20,000,000 *B. aertrycke*	98	60	61·3
3	Survived for 42 days after one feeding with 2,000,000 *B. aertrycke*	39	13	33·3
4	Survived for 42 days after three or four feedings with 2,000,000 *B. aertrycke*	59	39	66·1
5	Survivors from seven epidemics of *B. aertrycke* infection, each lasting three months	185	125	67·6

There seems little doubt that almost all mice, which survive the introduction of living *B. aertrycke* into their tissues, and the majority of those which either survive the per os administration of large or moderate doses, or are submitted to the risk of infection during any considerable epidemic of mouse typhoid, harbour *B. aertrycke* in their spleens over weeks or months, although they may give no evidence of the chronic infection from which they are suffering.

If we consider this fact in the light of the marked superiority, as an immunising agent, of living as contrasted with dead cultures of *B. aertrycke*, we can hardly escape the conclusion that the surviving mice are more resistant because they are suffering from a latent infection. We have, for instance, little doubt that the majority of the original selected group of 32 mice, in the experiment described above, were harbouring *B. aertrycke* in their tissues. It would seem that we are dealing with an example of the "depression" immunity which Morgenroth has described in his studies on superinfection (Morgenroth, 1920).

In this respect, then, our conclusions differ from Webster's. It appears to us most probable that the majority of normal mice, which he regarded as completely refractory to the per os administration of *B. aertrycke*, actually contracted a latent infection, and that to this factor they owed their resistance to the subsequent administration of this, or allied, organisms.

Our results do not, of course, in any way affect Webster's conclusions on the variation of resistance among a normal mouse population, nor do they detract from the fundamental importance of these results. We differ from him only in believing that those mice which are, *ab initio*, more resistant than their fellows, are probably not completely refractory to the administration of *B. aertrycke* in the dose which he employs, but respond by contracting a latent infection, which is compatible with prolonged survival and the entire absence of any evidence of disease, and that such mice are relatively resistant to further infection in consequence of this latent infection, and not merely by virtue of an initial natural resistance, which has allowed them to survive a preliminary selective process, in which their less resistant fellows were eliminated.

As regards the specificity or non-specificity of such immunity, our results clearly should not be regarded as incompatible with those recorded by Webster. He showed that increased resistance to *B. aertrycke* is associated with increased resistance to certain closely allied species of bacteria, to mercuric chloride, and to Botulinus toxin. Our own results would appear to demonstrate that increased resistance to *B. aertrycke* infection is not associated with increased resistance to infection with *Pasteurella*. They would appear to limit the significance of Webster's results, as showing that the high resistance of his surviving mice is not a generalised phenomenon, since it is not effective against all bacterial infections; but the findings are supplementary and not contradictory.

The study of the phenomenon of super-infection has not yet been pushed far enough to yield adequate data as regards the specificity of the reactions concerned. It would seem probable, *a priori*, that the degree of specificity would be less strict than in the case of that acquired resistance, which is associated with, if not dependent on, the appearance of specific antibodies in the blood. We should not, however, expect that a latent infection with a given bacterium would alter the resistance of the host to all other bacterial parasites. Such observations as have been recorded are in accord with these expectations, but the whole problem awaits further investigation. The evidence already available does, however, suggest that this type of resistance is of fundamental importance in the epidemic spread of bacterial infection.

REFERENCES.

AMOSS, H. L. (1922). The Effect of Addition of Healthy Mice to a Population suffering from Mouse Typhoid. *Journ. Exp. Med.* XXXVI. 45.

LOEFFLER, F. (1906). Ueber Immunisierung per os. *Leuthold Gedänkschrift*, I. 247.

MORGENROTH, J, BIBERSTEIN, H. and SCHNITZER, R. (1920). Die Depressionimmunität. *Deutsche med. Wochenschr.* XLVI. 337.

NEUFELD (1924). Ueber einige grundsätzliche Fragen der aktiven Immunisierung. *Zeitschr. f. Hyg.* CI. 468.

TOPLEY, W. W. C. (1921). The Potential Infectivity of a Surviving Mouse Population and their Resistance to Subsequent Epidemics of the same Disease. *Journ. of Hyg.* XX. 103.

TOPLEY, W. W. C. and WILSON, G. S. (1923). The Problem of Herd Immunity. *Ibid.* XXI. 243.

TOPLEY, W. W. C. and AYRTON, J. (1924). Further Investigations into the Biological Characteristics of *B. enteritidis* (*aertrycke*). *Ibid.* XXIII. 198.

TOPLEY, W. W. C., AYRTON, J. and LEWIS, E. R. (1924). Further Studies on an Experimental Epidemic of Mouse Typhoid. *Ibid.* XXIII. 223.

WEBSTER, L. T. (1923 *a*). Microbic Virulence and Host Susceptibility in Mouse Typhoid Infection. *Journ. Exp. Med.* XXXVII. 231.

—— (1923 *b*). The Virulence of an Epidemic Strain of *Bacillus pestis caviae*. *Ibid.* XXXVII. 781.

—— (1923 *c*). Microbic Virulence and Host Susceptibility in Paratyphoid enteritidis Infection of White Mice (1). *Ibid.* XXXVIII. 33.

—— (1923 *d*). Microbic Virulence and Host Susceptibility in Paratyphoid enteritidis Infection of White Mice (2). *Ibid.* XXXVIII. 45.

—— (1924 *a*). Microbic Virulence and Host Susceptibility in Paratyphoid enteritidis Infection of White Mice (3). *Ibid.* XXXIX. 129.

—— (1924 *b*). Microbic Virulence and Host Susceptibility in Paratyphoid enteritidis Infection of White Mice (4). *Ibid.* XXXIX. 879.

WEBSTER, L. T. and PRITCHETT, I. W. (1924). Microbic Virulence and Host Susceptibility in Paratyphoid enteritidis Infection of White Mice (5). *Ibid.* XL. 397.

WOLF, K. (1908). Immunisierung per os. *München. med. Wochenschr.* LV. 270.

YOSHIDA (1909). Ueber Immunisierung per os. *Arch. f. Hyg.* LXIX. 21.

(*MS. received for publication* 5. I. 1925.—Ed.)