FURTHER INVESTIGATIONS INTO THE BIOLOGICAL CHARACTERISTICS OF *B. ENTERITIDIS* (*AERTRYCKE*).

By W. W. C. TOPLEY AND JOYCE AYRTON[1].

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

WE recently reported in this *Journal* (Topley and Ayrton, 1924 *b*) the results obtained by administering cultures of *B. aertrycke* to mice, *per os*, and studying the subsequent excretion of this organism in the faeces. From these results we concluded that there was a marked correlation between the antigenic structure of a given strain of *B. aertrycke*, as revealed by agglutination, and the capacity of that strain to give rise to persistent faecal excretion. In a later report (Topley and Ayrton, 1924 *c*) we studied certain other characters of this bacterial species, which vary discontinuously, and concluded that there was definite evidence of linkage between certain pairs of attributes, so that both members of a pair were either present or absent in any one strain.

Between the date when the proofs of these reports were finally corrected and the actual date of publication, we obtained results which showed us that certain of our conclusions were rendered invalid by an unsuspected source of error in our technique. By the courtesy of the Editor, we were enabled to attach to each report an addendum, entering a caveat against the acceptance of our conclusions as there stated.

The present report deals with certain serological relations, which have been revealed by a re-investigation of the points at issue, and with the results obtained in a repetition of those of our earlier experiments which had been rendered meaningless by the error in question.

We have been largely concerned with the reaction of our bacterial strains to the type and group agglutinins, whose existence has been demonstrated by Andrewes (1922). Reference to the reports in question will show that a notably large proportion of all the strains of *B. aertrycke* which we examined agglutinated both with a type and with a group antiserum. We were led to the conclusion that such a reaction indicated the presence of both type and group antigen in the individual bacilli, rather than the presence through variation, of both type and group bacilli in the same culture; though the occurrence of such mixed cultures was, of course, recognised to be a common occurrence under certain circumstances. The argument involved the tacit assumption that the antisera employed were revealing the presence of type and group antigens, and of these alone. This assumption was false.

[1] A Report to the Medical Research Council.

The effect of the time and temperature of incubation of cultures of
B. aertrycke, on their subsequent agglutinability, has been referred to in earlier
reports; and the tendency for cultures, when incubated for a long time at
body temperature, to agglutinate with both group and type antisera has
been emphasised. The mixed agglutination in such cases was, however, re-
garded as evidence of bacterial variation, taking place during the later phase
of growth and multiplication.

In the course of a more recent experiment, in which numerous strains of
B. aertrycke were tested by agglutination, we noted that incubation, even at
22° C. for a period of 24 hours or more, or incubation at 37° C. for so short
a time as 16 hours, would result in a very high proportion of strains giving
mixed agglutination. We noted also that, when a batch of tests showed many
instances of mixed agglutination, strains reacting with the type serum alone
were usually absent, while strains reacting with group serum alone might be
present in considerable numbers. During the course of an experiment, in
which the faeces from a large number of mice were examined each day, a
high frequency of type strains, and an entire absence of mixed strains, would
suddenly be replaced by a high frequency of mixed strains, and an entire
absence of type strains, the relative frequency of the group strains being
unaffected. Reference to the records showed that the mixed agglutination
results occurred particularly with those cultures which were put up on a
Saturday, and which were incubated at 22° C. until the Monday morning, or,
in some instances, until the later hours of the Sunday. These observations
led us to test a considerable series of strains with regard to the effect of varia-
tion in the time and temperature of incubation on the subsequent agglutin-
ability of the cultures.

Agglutination Experiments.

The method adopted was as follows. Plates were inoculated with a given
strain of *B. aertrycke*, and were incubated over night. Next day several well
separated colonies were selected on each plate, a portion of each colony was
removed with a platinum loop, and, without recharging the loop, five or six
small tubes of broth were inoculated. These tubes were incubated for different
periods of time, and at different temperatures, and were then killed by the
addition of formalin and the application of heat, as described in earlier reports.
The killed cultures were then tested against type and group antisera.

Table I shows the results of one such series of tests, in which 258 colonies
were examined in this way.

The figures need little comment. Those strains which, when incubated
for 16 hours at 22° C. reacted with the type serum only, tended to react with
both sera when incubated for a longer time or at a higher temperature. So
marked was this tendency that after 16 hours at 37° C. over 97 per cent. of
the type strains had become so altered as to give mixed agglutination. Practi-
cally no difference was observed between those type strains which had been

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held in stock for several months, and frequently subcultured, and those type strains which had recently been isolated from the faeces or tissues of infected mice.

As regards those strains which, after incubation for 16 hours at 22° C. reacted with the group antiserum alone, prolongation of the time, or elevation of the temperature of incubation, had much less effect. The great majority reacted throughout with a group but not with a type serum. There was, however, some tendency for mixed agglutination to occur, and this was more in evidence with the old stock strains than with those recently isolated.

Up to this point, the results could have been accounted for by assuming that type strains of *B. aertrycke*, when their growth in broth culture was

Table I.

Showing agglutination results after growing B. aertrycke *for different times at different temperatures and testing against a monospecific type serum and a group serum (Newport).*

Type strains	No. of strains tested	16 hrs. at 22° C. Percentage of		16 hrs. at 37° C. Percentage of		24 hrs. at 22° C. Percentage of		24 hrs. at 37° C. Percentage of		40 hrs. at 22° C. Percentage of	
		T	M	T	M	T	M	T	M	T	M
Stock*	91	100	0	4·4	95·6	17·6	82·4	3·3	96·7	0	100
Recent	88	100	0	2·3	97·7	29·0†	71	0	100	4·5	95·5

Group strains	No. of strains tested	Percentage of		Percentage of		Percentage of		Percentage of		Percentage of	
		G	M	G	M	G	M	G	M	G	M
Stock*	34	100	0	73·5	26·5	82·4	17·6	79·4	20·6	85·3	14·7
Recent	45	100	0	93·3	6·7	100‡	0	100	0	100	0

T = Agglutination with *type* serum only.
G =   ,,   *group* ,,
M =   ,,   both sera.

* The *stock* strains had been cultivated on artificial media for several months, or years. The *recent* strains had been isolated from faeces or tissues within a few days or weeks of the agglutination tests.

† 69 strains tested.   ‡ 26 strains tested.

allowed to progress beyond a certain point, became altered in such a way as to react with group as well as with type agglutinin. Other series of tests, however, carried out in an exactly similar fashion, gave entirely different results. Cultures of type strains incubated at 37° C. for 48 hours or more, were agglutinated by the type serum alone, mixed agglutination being conspicuous by its absence.

It was obvious, from the grouping of the results, that some factor was involved common to all the tests of single series, and it was natural to examine more carefully the properties of the antisera employed. On the hypothesis put forward by Andrewes (1922), we had assumed that any antiserum, prepared against a member of the *B. paratyphosus* B group of bacteria, other than *B. aertrycke* (Mutton), would be a satisfactory source of group agglutinin acting on the latter organism. We had, by chance rather than by design, selected an

antiserum prepared against *B. aertrycke* (Newport), which agglutinated group strains of the Mutton bacillus to a high titre. We had, from time to time, substituted one batch of anti-Newport serum for another, when a given bottle was exhausted, each new batch being tested against several group strains of *B. aertrycke* (Mutton) to ensure the presence of an adequate amount of group agglutinin.

We now proceeded to test six such Newport sera, four paratyphosus B sera, four paratyphosus C sera, and three Gaertner sera against a group strain of *B. aertrycke* (Mutton), and against a type strain which had been grown at 37° C. for 40 hours. The results, set out in Table II, are somewhat surprising, but quite unequivocal. Clearly, the agglutination of the altered type strains, by

Table II.

Showing titres obtained by testing various sera against group strains of B. aer-
trycke grown at 22° *C. for* 16 *hours and against type strains grown at* 37° *C.*
for 40 *hours.*

	Bacterial suspensions	
	Group	Type
Serum*	16 hrs. at 22° C.	40 hrs. at 37° C.
Newport 1	6,400	1,600
„ 2	6,400	800
„ 3	6,400	200
„ 4	12,800	800
„ 5	1,600	0
„ 6	6,400	102,400
Paratyphosus B 1	3,200	0
„ 2	3,200	0
„ 3	1,600	0
„ 4	1,600	0
Paratyphosus C 1	800	0
„ 2	400	0
„ 3	400	0
„ 4	800	0
Gaertner 1	0	0
„ 2	0	0
„ 3	0	200

* No serum gave any agglutination with type strains of *B. aertrycke* grown at 22° C. for 16 hrs.

the Newport antisera, is unconnected with the presence of group agglutinin in the serum or of group antigen in the bacillus. An entirely different antigen-antibody complex has come into play. Five of the six Newport sera have a titre against the group strain of *B. aertrycke* which is practically constant, but their titre against the altered type strain varies over an enormous range. Serum Newport 3 and serum Newport 6 each agglutinates the group culture to a titire of 1/6400, but serum 3 agglutinates the altered type culture to 1/200, while serum 6 agglutinates it to 1/102,400. Again, the paratyphosus B sera contain considerable amounts of group agglutinin, but no trace of agglutinin for the altered type strain. The same is true for the paratyphosus C sera, though here the titres for the group strain are relatively low. The three Gaertner sera are almost without action, but it is of interest to note that,

while none of them contains group agglutinin, serum Gaertner 3 does agglutinate altered type strains to a low titre.

It is clear that type strains, under certain conditions of growth, to which in practice they are very commonly subjected, become altered in such a way that they react with an agglutinin to which they did not respond in their unaltered form.

It is of interest to determine whether or no group strains undergo a similar alteration. With the exception of the weakly acting Gaertner serum, those sera which reacted with altered type strains also contained group agglutinin in considerable amount, but an altered group strain could be distinguished from an unaltered group strain by a change in the titre at which it reacted. Using, for instance, a paratyphosus B serum, which agglutinates group strains of *B. aertrycke* to 1/6400, but does not react with altered type strains, and also

Table III.

Showing titres obtained by testing different group strains of B. aertrycke *against a paratyphosus B serum and a Newport serum, after growth for different times at different temperatures.*

	SERUM			
	Paratyphosus B		Newport 6	
Strain*	16 hrs. at 22° C.	48 hrs. at 37° C.	16 hrs. at 22° C.	48 hrs. at 37° C.
G 1	3,200	3,200	8,000	64,000
G 2	3,200	3,200	8,000	32,000
G 3	3,200	3,200	8,000	64,000
G 4	1,600	3,200	4,000	64,000
G 5	1,600	3,200	4,000	128,000
G 6	1,600	3,200	4,000	128,000
G 7	3,200	1,600	8,000	128,000
G 8	1,600	1,600	4,000	64,000
G 9	3,200	3,200	8,000	128,000
G 10	3,200	3,200	8,000	128,000
G 11	1,600	3,200	4,000	64,000
G 12	1,600	1,600	4,000	128,000

* All strains were tested against a type serum with negative results.

serum Newport 6, which has a titre of 1/6400 for group strains and 1/102,400 for altered type strains, we can grow group strains for various times at various temperatures and then test them against these two sera. If we find that a group strain, when grown under those conditions which produce antigenic alterations of the type strain, reacts with the Newport 6 serum to a markedly higher titre than 1/6400, while its agglutinability by the paratyphoid B serum is unchanged, then it would seem a safe conclusion that both type and group strains undergo the same antigenic alteration in broth cultures. Table III shows that these conditions do in fact hold true. It would clearly be possible to obtain additional information on this point by adequate absorption tests.

We may then conclude that we have been dealing with two different phenomena. In addition to the type and group antigen, and the corresponding agglutinins, there is another antigen constituent, which for the moment we may refer to as the X antigen with its corresponding X agglutinin. Broth

cultures derived from a type or group colony of the same organism and grown under suitable conditions contain type or group antigen alone, except in so far as, in either case, a few bacilli of the alternative serological variety may be present. If the time of incubation be prolonged, or the temperature be raised, the X antigen begins to appear in either the type or the group culture, and is thereafter present in addition to the type or group antigen.

From the results of the present series of experiments it would appear that the distribution of type agglutinin, group agglutinin and X agglutinin is somewhat as follows:

(*a*) A serum prepared against *B. aertrycke* (Mutton), and absorbed with other organisms of the same general bacterial group, contains type agglutinin, but neither group agglutinin nor X agglutinin.

(*b*) A group serum, *i.e.* a serum reacting with young broth cultures of group strains of *B. aertrycke* (Mutton), appears to vary according to the organism against which it is prepared.

(*c*) Sera prepared against group strains of *B. paratyphosus* B contain group agglutinin, but no X agglutinin and *ex hypothesi* no type agglutinin for *B. aertrycke* (Mutton).

(*d*) Sera prepared against group strains of *B. paratyphosus* C also contain the group agglutinin alone.

(*e*) Sera prepared against group strains of *B. aertrycke* (Newport) contain group agglutinin, and may or may not contain X agglutinin. The amount of X agglutinin in different batches of anti-Newport serum varies over an enormous range and shows no constant relation to the amount of group agglutinin.

(*f*) Sera prepared against *B. Gaertner* contain no group agglutinin, but may contain some X agglutinin.

The question of the presence or absence of X agglutinin in type sera in general clearly needs further study. It would be natural to suppose that a serum prepared against a type strain, which had undergone antigenic alteration, would contain X agglutinin. This X agglutinin might well be removed by subsequent absorption with closely related bacteria which had themselves developed the X antigen. We have, however, made no attempt to pursue this aspect of the question[1].

What relation this X agglutinin may bear to the para-agglutinins or neben-agglutinins of certain workers, we need not now enquire. Clearly it is fundamentally distinct from the type and group agglutinins. So far as our results have significance, in this respect, they are in complete accord with the views which Andrewes has put forward.

It was clear that the occurrence of mixed agglutination in the broth cultures, with which we had been dealing, had entirely misled us with regard

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[1] The results of some recent observations, not connected with the present investigation, suggest that the phenomenon of mixed agglutination with cultures grown at 37° C. occurs with many organisms of the paratyphoid-enteritidis group of bacilli.

to the nature of the viable bacteria which those cultures contained. It seemed desirable to determine, as far as possible, the actual constitution of cultures which agglutinated with type serum, with group serum or with both.

An analysis of this kind was attempted by taking broth cultures, which had been tested by agglutination after growth at 22° C. for 16 hours, and preparing plate cultures from them. Next day 20 to 50 colonies from each such plate were subcultured into tubes of broth, these were incubated for 16 hours at 22° C., killed and tested by agglutination. Table IV shows the results obtained with 30 type strains, 30 group strains and 25 mixed strains. The results are quite clear-cut. Under such conditions, a culture agglutinating with a type serum alone gives rise to over 99 per cent. of type colonies when plated, a culture agglutinating with a group serum alone gives rise to over 95 per cent. of group colonies; while a mixed culture gives rise to both type and group colonies in not widely differing proportions, and mixed colonies

Table IV.

Showing results obtained by plating from cultures, which had been tested by agglutination after incubation at 22° C. for 16 hours, and determining the agglutination reaction of many colonies from each plate.

Agglutination of original culture	Number of cultures tested	Number of colonies tested*	Percentage of colonies agglutinating as		
			Type	Group	Mixed
Type	30	1464	99·5	0·4	0·1
Group	30	1465	2·7	95·2	2·1
Mixed	25†	464	36	53·9	10·1

* 50 colonies were subcultured from each of the type or group plates: 20 from each of the mixed plates. A few colonies failed to give growth in subculture.

† 21 of the 25 mixed strains gave both type and group colonies on the same plate.

are relatively frequent. It seems clear that those cultures, which react with both type and group sera after growth at 22° C. for 16 hours, do so because they contain both type and group bacilli. The difference between the figures obtained with the type and group strains, though small, is, we believe, significant. It will be noted that among the colonies derived from type cultures only 0·4 per cent. react as group, while among the colonies derived from group cultures 2·7 per cent. reacted as type. This difference was very consistent over the 60 strains examined. For instance, 25 of the 30 type strains yielded colonies which were all type, while 12 only of the 30 group strains yielded colonies which were all group. We shall return to this difference later.

If now we turn to those cultures which give mixed agglutination after growing at 37° C. for 16 hours or longer, but which are known to have reacted during an earlier phase of growth as pure type strains, we obtain very different results. Table V gives such results with regard to 13 cultures. It is clear that the proportion of type colonies derived from a culture which has changed from type to mixed, as judged by agglutination, during relatively prolonged growth, does not differ sensibly from the proportion of type colonies derived from a culture which gives pure type agglutination. It would seem that the

X antigen first appears in those bacilli which are dead or dying, and so are unable to multiply further; or alternatively that, when an altered type bacillus containing the X antigen undergoes further division, it reverts to the non-X containing form.

It is clear that we may very easily avoid the source of error introduced by this antigenic alteration, by adhering strictly to 16 hours as the time, and 22° C. as the temperature of incubation, and that under these circumstances any group antiserum may be employed. Or, alternatively, we may select a group serum which contains no X agglutinin, and under these circumstances the time and temperature of incubation of the broth cultures may be varied within relatively wide limits.

When we adopt such precautions we find that cultures showing mixed agglutination are relatively very infrequent. During the past four and a half months we have tested 12,407 strains of *B. aertrycke*, recently isolated from the faeces or tissues of mice. Of these 7519 or 60·6 per cent. reacted as type,

Table V.

Showing results obtained by plating from cultures which gave mixed agglutination after incubation for 16 hours or more at 37° C., but which had reacted as type after 16 hours at 22° C., and testing many colonies from each plate by agglutination.

Number of strains	Number of colonies tested	Percentage of colonies agglutinating as		
		Type	Group	Mixed
13	239	98·8	0·8	0·4

4672 or 37·7 per cent. as group, and 216 or 1·7 per cent. as mixed. We have shown reasons for believing that those strains which, under these conditions, give mixed agglutination contain bacilli of each serological variety.

The results here reported offer an adequte explanation of the difference between our previous findings and those recorded by Andrewes, who claimed that the great majority of strains he examined reacted sharply either as type or group when tested by agglutination. Had we chanced to select any other group serum than that actually employed, we should have obtained the same results. The figures recorded above are entirely in accord with Andrewes' hypothesis.

It would seem, also, that variation from type to group, or *vice versa*, in artificial culture, is not such a rapid, irregular and unpredictable phenomenon as we had supposed. Table VI shows the results obtained in a short series of tests designed to yield information on this point. Eight sets of cultures were examined, four of type and four of group strains. In series T 1 and G 1, broth cultures of type and group strains respectively were continuously incubated at 22° C. and broth subcultures were daily prepared from them, grown for 16 hours at 22° C. and tested by agglutination. In series T 2 and G 2 similar broth cultures were daily subcultured to fresh broth, and then killed and submitted to agglutination: thus, this series represents a chain, or succession

of subcultures in broth. Series T 3 and G 3 show the results of continuous incubation on agar, daily subcultures being made into broth for agglutination. Series T 4 and G 4 show the result of daily subculture from agar to agar, with the inoculation of parallel broth cultures from each agar tube, for the agglutination tests.

The figures given in the table represent the number of strains, examined on the day in question, which gave agglutination with both antisera, denoting that bacilli, of the alternative serological variety to that of which the original culture was composed, had begun to put in an appearance. Thus, on the sixth day, one of the 10 type strains subjected to prolonged cultivation on agar, gave rise to group bacilli, as well as type bacilli, on subculture; while eight out of nine group strains, which had been subjected to daily subculture from agar to agar, gave rise to both type and group bacilli. We should not attach any great significance to the results of this experiment, since the total number of strains included is small, but a few points seem clearly established. Within

Table VI.

Showing the number of cases in which group variants appeared in type cultures, or vice versa, *under various conditions of growth.*

		No. of strains	DAY								
	Series		1	2	3	4	5	6	7	8	9
T 1.	Broth continuous	13	0	0	0	0	0	—	0	0	0
T 2.	„ subcultures	13	0	0	0	0	0	—	0	1	0
T 3.	Agar continuous	10	0	0	0	0	—	1	0	0	—
T 4.	„ subcultures	10	0	0	0	0	—	0	0	0	—
G 1.	Broth continuous	7	0	0	0	0	0	—	1	2	3
G 2.	„ subcultures	7	0	0	0	0	0	—	0	1	—
G 3.	Agar continuous	9	0	0	0	0	—	0	0	0	—
G 4.	„ subcultures	9	0	0	0	6	—	8	9	9	—

three days there is no evidence of serological variation in any case. Considering broth cultures alone there is no evidence of any serological variation within five days. The results of this experiment are in entire agreement with the view, based on the general results of many thousand tests, that, by subculturing a given colony to broth, killing the broth culture after a suitable period of incubation and testing it against the appropriate sera, we may obtain accurate information as to the character of the bacilli composing the colony in question.

It is clear from the table that the frequency with which type strains give rise to group variants is less than the frequency with which group strains give rise to type variants. In this connection we may recall that analysis of cultures, reacting as type and group respectively, showed that the frequency of occurrence of group bacilli in type cultures was less than the frequency of occurrence of type bacilli in group cultures. The table seems also to afford evidence that repeated subculture on agar is the most effective method of promoting serological variation. It seems possible that this may result from the increased probability of selecting chance variants, when some portion of a surface growth

is picked at random for subculture and this process repeated at frequent intervals.

THE EXCRETION OF *B. AERTRYCKE* IN THE FAECES.

In our previous studies on the faecal excretion of *B. aertrycke*, we were led to correlate the presence of group antigen with persistent faecal excretion. Since our method of determining the presence of group antigen was quite untrustworthy, our conclusions on this point as published are valueless.

It seems probable that the great majority of those strains which gave mixed agglutination were in reality altered type strains, but little purpose would be served by attempting any detailed analysis of our earlier results in the light of after knowledge. We may, however, safely make one assumption. In certain experiments we fed type strains to mice, and because we repeatedly isolated from the faeces strains which gave mixed agglutination, we assumed that we had failed in our object, and included those experiments with those in which group or mixed strains had been fed to the mice. Clearly this was

Table VII.

Showing corrected figures for experiments recorded in a previous report.

	Group strains No correction required	Type strains	
		As previously recorded	Corrected
Number of mice examined	35	35	50
Number of mice excreting *B. aertrycke*	20	4	14
Percentage of mice excreting *B. aertrycke*	57·1	11·4	28
Number of specimens examined	438	458	635
Number of specimens positive	70	6	42
Percentage of specimens positive	16	1·3	6·8

unjustified. We must classify these earlier experiments on the basis of the serological results obtained with the cultures used for feeding, irrespective of the agglutination results with the strains isolated from the faeces or from the tissues. If we do this we obtain a very different picture. Taking the figures given in Table I of the report referred to (Topley and Ayrton, 1924 *b*), we should transfer experiments *S* and *P* to the type class. The results of this transference are shown in Table VII, where the figures are compared with those set out in Table II of the earlier report. The figures, as emended, still suggest that feeding on group strains is more often followed by persistent excretion in the faeces, than is feeding on type strains; but there is not the sharp and apparently discontinuous variation which appeared to exist on the earlier basis of classification.

Clearly, however, it was necessary to repeat these experiments, using a technique free from the source of error which we had detected.

The general technique adopted did not differ from that already described (Topley and Ayrton, 1924 *a* and *b*) and it will suffice to recall a few of the more important details.

The broth cultures of *B. aertrycke* were administered by allowing drops to fall from a calibrated dropping-pipette into the open mouth of the mouse. Each mouse was subsequently housed in a separate cage, and observed for 42 days, or until its death if this occurred earlier. Specimens of faeces were examined on the 2nd and 3rd days after feeding, three times during each of the succeeding two weeks, twice during each of the three following weeks, and on the 41st and 42nd days. The number of viable *B. aertrycke* present was estimated by the method already described, and recorded on a logarithmic scale. An Excretion Coefficient, for any given series of specimens, collected from any series of mice, was calculated by dividing the total score recorded by the number of specimens in the series, and multiplying the result by 100.

All mice which died were examined post-mortem. Cultures were taken from the heart and spleen and, where a growth of *B. aertrycke* was obtained, plate cultures were prepared and 20 colonies from each plate were tested by agglutination. All surviving mice were killed on, or shortly after, the 42nd day. A portion of the spleen of each mouse was transferred to a tube of broth, these tubes were incubated for 48 hours, at 37° C., and any tube which showed growth was plated and examined in the same way as were the spleen cultures from those mice which succumbed to infection.

Tables VIII to XIII give a summary of the results obtained. Each table summarises four to five experiments. In each experiment five mice were fed on a type or a group strain. A few of these mice died before any faecal examination was made, and they are not included in the tables.

Tables VIII and IX refer to ten experiments in five of which type strains, and in five group strains were administered to mice, *per os*, in a single dose of

Table VIII.

Showing results of feeding one large dose of type strain of
B. aertrycke.

Number of mice fed	29
Number of mice which excreted *B. aertrycke*	16
Percentage of mice which excreted *B. aertrycke*	55·2
Number of specimens of faeces examined	284
Number of specimens positive	44
Percentage of specimens positive	15·5
Excretion coefficient	64·4
Percentage of colonies from faeces reacting as ⎰ Type	84·0
⎱ Group	13·3
⎱ Mixed	2·7
Number of mice which died	16
Number of mice positive on post-mortem examination	16
Specific mortality per cent.	55·2
Percentage of colonies from tissues of dead mice reacting as ⎰ Type ...	93·8
⎱ Group ...	5·3
⎱ Mixed ...	0·9
Number of survivors killed on 42nd day	13
Number of survivors with positive spleen cultures	7
Percentage of survivors with positive spleen cultures	53·8
Percentage of colonies from spleen cultures reacting as ⎰ Type... ...	98·6
⎱ Group ...	0
⎱ Mixed ...	1·4

Table IX.

*Showing results of feeding one large dose of a group strain of
B. aertrycke.*

Number of mice fed		24
Number of mice which excreted *B. aertrycke*		17
Percentage of mice which excreted *B. aertrycke*		70·8
Number of specimens of faeces examined		289
Number of specimens positive		50
Percentage of specimens positive		17·3
Excretion coefficient		69·5
Percentage of colonies from faeces reacting as { Type		21·6
{ Group		75·6
{ Mixed		2·8
Number of mice which died		7
Number of mice positive on post-mortem examination		7
Specific mortality per cent.		29·2
Percentage of colonies from tissues of dead mice reacting as { Type ...		1·4
{ Group ...		81·4
{ Mixed ...		17·2
Number of survivors killed on 42nd day		17
Number of survivors with positive spleen cultures		13
Percentage of survivors with positive spleen cultures		76·5
Percentage of colonies from spleen cultures reacting as { Type... ...		80·4
{ Group ...		17·3
{ Mixed ...		2·3

0·04 c.c. of an 18 hour broth culture, grown at 22° C. Tables X and XI
summarise 10 experiments, in which a single dose of 1/20th of this amount
was employed, and Tables XII and XIII summarise eight experiments in
which the latter dose, 0·002 c.c. was repeated on four separate occasions.

In these experiments the group strains did not lead to a markedly greater
degree of faecal excretion than did the type strains. Some difference is
apparent, and it is always a difference in this direction, but it is far less

Table X.

*Showing results of feeding one small dose of type strain of
B. aertrycke.*

Number of mice fed		25
Number of mice which excreted *B. aertrycke*		4
Percentage of mice which excreted *B. aertrycke*		16
Number of specimens of faeces examined		361
Number of specimens positive		9
Percentage of specimens positive		2·5
Excretion coefficient		10·5
Percentage of colonies from faeces reacting as { Type		100
{ Group		0
{ Mixed		0
Number of mice which died		5
Number of mice positive on post-mortem examination		5
Specific mortality per cent.		20
Percentage of colonies from tissues of dead mice reacting as { Type ...		95
{ Group ...		5
{ Mixed ...		0
Number of survivors killed on 42nd day		20
Number of survivors with positive spleen cultures		6
Percentage of survivors with positive spleen cultures		30
Percentage of colonies from spleen cultures reacting as { Type... ...		71·7
{ Group ...		25·8
{ Mixed ...		2·5

Table XI.

Showing results of feeding one small dose of a group strain of
B. aertrycke.

Number of mice fed		24
Number of mice which excreted *B. aertrycke*		7
Percentage of mice which excreted *B. aertrycke*		29·2
Number of specimens of faeces examined		333
Number of specimens positive		14
Percentage of specimens positive		4·2
Excretion coefficient		17·1
Percentage of colonies from faeces reacting as	Type	14·3
	Group	85·7
	Mixed	0
Number of mice which died		5
Number of mice positive on post-mortem examination		5
Specific mortality per cent.		20·8
Percentage of colonies from tissues of dead mice reacting as	Type ...	31·4
	Group ...	64·4
	Mixed ...	4·2
Number of survivors killed on 42nd day		19
Number of survivors with positive spleen cultures		7
Percentage of survivors with positive spleen cultures		36·8
Percentage of colonies from spleen cultures reacting as	Type... ...	47·6
	Group ...	50·2
	Mixed ...	2·2

clear-cut and consistent than appeared to be the case in our earlier experiments, even when allowance was made for faulty technique. The figures which afford data for comparison in Tables VIII to XIII are the percentage of mice excreting *B. aertrycke*, the percentage of specimens of faeces yielding positive results, and the excretion coefficient. When one large dose of culture was administered (Tables VIII and IX) the figures for these values are all higher for the group than for the type series, though the differences are slight and of

Table XII.

Showing results of feeding with repeated (4) *small doses of type strains of* B. aertrycke.

Number of mice fed		20
Number of mice which excreted *B. aertrycke*		11
Percentage of mice which excreted *B. aertrycke*		55
Number of specimens of faeces examined		308
Number of specimens positive		40
Percentage of specimens positive		13
Excretion coefficient		44·1
Percentage of colonies from faeces reacting as	Type	98·5
	Group	1
	Mixed	1·5
Number of mice which died		4
Number of mice positive on post-mortem examination		4
Specific mortality per cent.		20
Percentage of colonies from tissues of dead mice reacting as	Type ...	95·6
	Group ...	3·1
	Mixed ...	1·3
Number of survivors killed on 42nd day		16
Number of survivors with positive spleen cultures		7
Percentage of survivors with positive spleen cultures		43·75
Percentage of colonies from spleen cultures reacting as	Type	97·9
	Group ...	2·1
	Mixed ...	0

Table XIII.

Showing results of feeding repeated (4) *small doses of group strains of* B. aertrycke.

Number of mice fed	20
Number of mice which excreted *B. aertrycke*	11
Percentage of mice which excreted *B. aertrycke*	55
Number of specimens of faeces examined	298
Number of specimens positive	48
Percentage of specimens positive	16·1
Excretion coefficient	56
Percentage of colonies from faeces reacting as { Type	13
Group	83·75
Mixed	3·25
Number of mice which died	4
Number of mice positive on post-mortem examination	3
Specific mortality per cent.	15
Percentage of colonies from tissues of dead mice reacting as { Type	42
Group	55·3
Mixed	2·7
Number of survivors killed on 42nd day	16
Number of survivors with positive spleen cultures	9
Percentage of survivors with positive spleen cultures	56·25
Percentage of colonies from spleen cultures reacting as { Type	37·8
Group	61·5
Mixed	0·7

doubtful significance. In Tables X and XI, giving the results which followed the administration of one small dose of *B. aertrycke*, the differences are rather more marked, though the total number of positive results, upon which comparisons depend, is smaller throughout. With repeated small doses (Tables XII and XIII) the difference in the percentage of mice excreting, as between those fed on type and group strains, disappears. The percentage of specimens positive and the excretion coefficient are, however, higher for the group than for the type series. This indicates that, with repeated infection, while the proportion of mice which excrete *B. aertrycke* on one occasion at least is the same among those fed on type as among those fed on group strains; yet, among the latter, those mice which excrete tend to do so more persistently. The differences are so small that we should attach little significance to them, were it not for their entire consistency. When all the experiments are considered together, it seems to us that they do afford some evidence in favour of an increased frequency of faecal excretion following the administration of group strains as compared with type strains of *B. aertrycke*.

In calculating the proportions of the two serological varieties recovered from the faeces or from the tissues, under different conditions, certain adjustments have necessarily been made. It has been our routine procedure to subculture five colonies of *B. aertrycke* from each faecal culture, when that number were available. On many occasions we have examined larger numbers of colonies, but frequently less than five colonies of *B. aertrycke* have appeared on the primary cultures from the faeces. Each specimen examined should clearly exert an equal influence on the calculated results. For this reason we have allocated a total value of 5 to the agglutination results obtained with

any one specimen of faeces, this figure being divided in accordance with the results obtained. From the tissues of mice which have died from infection, we have examined 40 colonies, 20 being taken from the heart culture and 20 from the spleen culture. From those mice which survived the experimental period and were subsequently killed, only 20 colonies have been examined, since spleen cultures alone were obtained. In a small proportion of cases some of the colonies from the plate have failed to grow in subcultures. In con-

Table XIV.

Showing results of inoculating mice with small doses of type strains of B. aertrycke, *intraperitoneally and subcutaneously.*

Number of mice inoculated	19
Number of mice which excreted *B. aertrycke*	5
Percentage of mice which excreted *B. aertrycke*	26·3
Number of specimens of faeces examined	120
Number of specimens positive	6
Percentage of specimens positive	5
Excretion coefficient	15·8
Percentage of colonies from faeces reacting as {Type	83·3
Group	0
Mixed	16·7
Number of mice which died	7
Number of mice positive on post-mortem examination	7
Specific mortality per cent.	36·8
Percentage of colonies from tissues of dead mice reacting as {Type ...	99
Group ...	0·7
Mixed ...	0·3
Number of survivors killed on 42nd day	12
Number of survivors with positive spleen cultures	9
Percentage of survivors with positive spleen cultures	75
Percentage of colonies from spleen cultures reacting as {Type	83·9
Group ...	13·9
Mixed ...	2·2

Table XV.

Showing results of inoculating mice with small doses of group strains of B. aertrycke, *intraperitoneally or subcutaneously.*

Number of mice inoculated	17
Number of mice which excreted *B. aertrycke*	6
Percentage of mice which excreted *B. aertrycke*	35·3
Number of specimens of faeces examined	119
Number of specimens positive	19
Percentage of specimens positive	16
Excretion coefficient	60·5
Percentage of colonies from faeces reacting as {Type	10·1
Group	87·2
Mixed	2·7
Number of mice which died	5
Number of mice positive on post-mortem examination	5
Specific mortality per cent.	29·4
Percentage of colonies from tissues of dead mice reacting as {Type ...	42·5
Group ...	37
Mixed ...	20·5
Number of survivors killed on 42nd day	12
Number of survivors with positive spleen cultures	11
Percentage of survivors with positive spleen cultures	91·7
Percentage of colonies from spleen cultures reacting as {Type	49·5
Group ...	48·6
Mixed ...	1·9

formity with the procedure adopted with the faecal cultures, we have allocated a total figure of 20 for the agglutination results, obtained with the cultures from any one mouse, in calculating the proportion of the two serological varieties isolated from the tissues.

We append summaries (Tables XIV and XV) of two small series of experiments, in which the faecal excretion of B. aertrycke was studied in mice which had been injected subcutaneously or intraperitoneally with small doses of this organism. These results appear to us to yield considerable support to the view that group strains of B. aertrycke are in some way better endowed than are type strains for multiplication in the intestinal tract.

If now we compare the proportion of the two serological varieties isolated from the faeces and from the tissues of dying or surviving mice, we find ample confirmation of the differences noted in our earlier experiments. In Tables VIII, X and XII, the percentages of type strains isolated from the faeces were 84, 100 and 98·5 per cent., from the tissues of mice which died 93·8, 95 and 95·6 per cent., and from the tissues of those mice which survived beyond the 42nd day 98·6, 71·7 and 97·9 per cent. In general, then, when mice are fed on type strains, the strains recovered from their faeces or from their tissues after death belong to the same serological variety.

In Tables IX, XI and XIII, the percentage of type strains are, for those of faecal origin, 21·6, 14·3 and 13 per cent., for those from the tissues of dead mice 1·4, 31·4 and 42 per cent., for those from the tissues of mice surviving beyond the 42nd day, 80:4, 47·6 and 37·8 per cent. In general then, when mice are fed on group strains, a certain proportion of type strains are recovered from the faeces and from the tissues; but, while the group bacilli markedly outnumber the type bacilli in the faeces, the proportion of type bacilli in the tissues may equal or exceed the proportion of group bacilli, and the relative frequency of the type strains in the tissues is greater in the case of surviving mice than in the case of those which succumb to infection.

A glance at Tables XIV and XV will show that similar facts hold true.

In Tables XVI and XVII are collected figures showing the relation between the proportion of type and group strains isolated from the faeces or tissues of any mouse, and the interval between the last administration of B. aertrycke and the day on which the strain was isolated. While there is little variation with any of the type series, the group series show a tendency for the group strains to be replaced by type strains to an increasing extent with lapse of time.

It would seem that mice which receive relatively large doses of group bacilli, either by the mouth or parenterally, and hence succumb rapidly to a general tissue invasion, yield in most cases strains of the same serological type from their tissues. If, however, the infection produced be subacute or chronic, then the majority of the group bacilli are eventually replaced by the type variety.

This progressive replacement of one serological variety by another may be

due to some selective action of the tissues, tending to the preservation of type strains and the elimination of group strains of *B. aertrycke*. It may be that the tendency for the proportion of type strains in the faeces of mice, which have been fed on group strains of *B. aertrycke*, to increase with the interval which elapses between the feeding and the collection of a specimen of faeces for examination, may be a reflection of the selective action of the tissues from the sphere of whose influence the bacilli are returning to the intestinal tract.

Table XVI.

Showing the percentage of type, group and mixed strains isolated from the faeces of mice fed on type or group strains of B. aertrycke, *according to the time which had elapsed since feeding.*

	Strains administered						
	Group				Type		
Day	No. of specimens	Type	Group	Mixed	No. of specimens	Type	Group	Mixed
1–5	31	6·8	92·6	0·6	14	89·7	0	10·3
6–10	26	13·5	79·6	6·9	15	92·8	7·2	0
11–15	13	7·7	92·3	0	12	76·7	20·0	3·3
16–21	13	21·5	75·4	3·1	11	100	0	0
22–42	19	41	55·3	3·7	27	96·3	3	0·7

Table XVII.

Showing the percentage of type, group and mixed strains isolated from the tissues of mice fed on or inoculated with, type or group strains of B. aertrycke, *according to the time which had elapsed between administration of the bacteria and the death of the mice.*

	Strains administered						
	Group				Type		
Day	No. of mice	T	G	M	No. of mice	T	G	M
1–5	23	8·9	84·8	6·3	16	97·2	1·2	1·6
6–10	14	21·1	67·1	11·8	15	98·3	1·0	0·7
11–15	8	21·25	67·5	11·25	13	95·4	3·1	1·5
16–21	19	38·7	50·8	10·5	19	89·5	9·7	0·8
22–42	44	56·8	37·0	6·2	40	92·5	6·5	1·0

We must, however, consider the possibility that such results as those recorded above are the expression of an innate tendency for group strains to give rise to type variants, more readily than type strains give rise to group variants. We have already recorded experiments which suggest that such a tendency is, in fact, observable in cultures of *B. aertrycke* grown on the ordinary media of the laboratory.

The balance of probability is, perhaps, in favour of some selective action on the part of the tissues. In many cases mice, fed on group strains, have yielded spleen cultures giving only type bacilli on plating. Thus we must explain in some way the apparent disappearance of the group bacilli as well

as the appearance of the type bacilli. Again, there is no evidence, when type strains are fed to mice, that there is a tendency for group strains to appear in the tissues, and gradually to replace the type strains. The rate of production of variants by the two serological varieties in artificial culture does not appear to differ so widely as would be suggested by the results obtained *in vivo*. To account for these, apart from a selective action of the tissues, we should have to assume that the frequency of occurrence of group variants in type strains of *B. aertrycke* was of an almost negligible order.

It must, however, be remembered that we have not in these experiments employed cultures derived from single bacterial cells. Until we have more exact knowledge of the rate and direction of this particular type of bacterial variation, under many different conditions, we lack an essential part of the data required for arriving at a conclusion on the point at issue.

The minimal lethal dose of cultures of the two serological varieties, and of rough and smooth variants.

In our previous experiments, the serological reaction of different smooth strains of *B. aertrycke* was found to be uncorrelated with the minimal lethal dose of these strains, as judged by intraperitoneal injections. This finding has been confirmed in the experiments summarised in Tables XVIII and XIX.

The greatly increased minimal lethal dose of rough as compared with smooth strains, referred to in our previous report (Topley and Ayrton, 1924 *b*), was not invalidated by the error in our serological technique, and it appeared unnecessary to repeat these experiments.

Table XVIII.

Showing results of inoculating mice with an 18 hours' broth culture of B. aertrycke.

(Type.)

Dose in c.c.	Route	No. of mice	No. of mice died	Day of death	No. of mice survived	Percentage of mice survived
0·25	I.P.	2	2	1, 1	0	0
0·025	,,	2	2	1, 1	0	0
0·0025	,,	4	3	1, 5, 10	1	25
0·00025	,,	4	4	1, 5, 6, 9	0	0
0·000025	,,	4	1	1	3	75
0·0000025	,,	2	1	7	1	50
0·00000025	,,	2	0	—	2	100
0·25	Sc.	2	2	2, 5	0	0
0·025	,,	2	2	4, 5	0	0
0·0025	,,	4	3	2, 9, 6	1	25
0·00025	,,	4	3	7, 10, 14	1	25
0·000025	,,	4	3	12, 13, 19	1	25
0·0000025	,,	2	0	—	2	100
0·00000025	,,	2	0	—	2	100

Total number of mice inoculated = 40.   I.P. = intraperitoneal.
     ,,   ,,   died   = 26.   Sc. = subcutaneous.
Percentage mortality   = 65.

14—2

Table XIX.

Showing results of inoculating mice with an 18 *hours' broth culture*
of B. aertrycke.

(Group.)

Dose in c.c.	Route	No. of mice	No. of mice died	Day of death	No. of mice survived	Percentage of mice survived
0·25	I.P.	2	2	1, 4	0	0
0·025	,,	2	2	3, 4	0	0
0·0025	,,	4	4	2, 3, 5, 5	0	0
0·00025	,,	4	3	5, 5, 7	1	25
0·000025	,,	4	1	2	3	75
0·0000025	,,	2	2	5, 8	0	0
0·00000025	,,	2	0	—	2	100
0·25	Sc.	2	2	4, 5	0	0
0·025	,,	2	2	4, 7	0	0
0·0025	,,	4	3	2, 4, 11	1	25
0·00025	,,	4	2	16, 17	2	50
0·000025	,,	4	2	7, 9	2	50.
0·0000025	,,	2	0	—	2	100
0·00000025	,,	2	1	7	1	50

Total number of mice inoculated = 40.
,, ,, died = 26.
Percentage mortality = 65.

In the report already referred to, it was noted that the specific mortality following administration *per os,* did not differ significantly as between smooth type and smooth group strains, while the specific mortality following the administration of rough variants was definitely lower than when smooth strains were employed.

It will be noted that the figures recorded in Tables VII and IX of the present report suggest that the oral administration of type strains is followed by a higher mortality than is the oral administration of group strains. The actual course of events in the individual experiments summarised in these tables suggested that the apparent excess of mortality was due to an excessive death-rate in two small series of mice, and was probably not significant. To test this point further, two larger series of mice were fed on the same amount of culture, and the subsequent course of events was observed for 42 days, without examination of the faeces. The results are recorded in Tables XX and XXI, and show a slightly greater specific mortality with the group strains.

We may then accept the conclusion that the lethal effect of the administration of cultures of *B. aertrycke* varies sharply with the roughness or smoothness of the strain employed, but is uncorrelated with the presence of type or group antigen.

It should, perhaps, again be emphasised that, as we have never isolated rough strains during the course of experimental epidemics of mouse-typhoid, it is impossible to judge what rôle, if any, rough strains may play in the natural spread of infection.

Table XX.

Showing results obtained by feeding 50 mice on type strains of B. aertrycke, one large dose (0·02 c.c. of an 18 hours' broth culture) being administered.

Number of mice fed	50
Number of mice which died	17
Percentage of mice which died	34
Number of mice positive on post-mortem examination	11
Specific mortality per cent.	22

Percentage of colonies from tissues of dead mice reacting as	Type ...	90·9
	Group ...	5·9
	Mixed ...	3·2

Number of survivors killed on 42nd day	33
Number of survivors with positive spleen cultures	19
Percentage of survivors with positive spleen cultures	57·6

Percentage of colonies from spleen cultures reacting as	Type	96·6
	Group ...	3·4
	Mixed ...	0

Table XXI.

Showing results obtained by feeding 50 mice on group strains of B. aertrycke, one large dose (0·02 c.c. of an 18 hours' broth culture) being administered.

Number of mice fed	50
Number of mice which died	15
Percentage of mice which died	30
Number of mice positive on post-mortem examination	14
Specific mortality per cent.	28

Percentage of colonies from tissues of dead mice reacting as	Type ...	16·4
	Group ...	66·3
	Mixed ...	17·3

Number of survivors killed on 42nd day	35
Number of survivors with positive spleen cultures	21
Percentage of survivors with positive spleen cultures	60

Percentage of colonies from spleen cultures reacting as	Type	42·9
	Group ...	46·7
	Mixed ...	10·4

DOSAGE.

So far as the question of dosage is concerned, the general results of the present series of experiments do not differ from those already recorded. We have not, however, repeated the feeding experiments with progressively decreasing amounts of culture, so that we have not covered the same range of dosage as in our earlier studies.

From the figures given in Tables VIII to XI, it will be seen that the larger the dose of culture administered the higher is the proportion of mice which excrete *B. aertrycke* in their faeces, the greater the death-rate, and the greater the proportion of surviving mice which harbour *B. aertrycke* in their tissues. With repeated doses (Tables XII and XIII) together amounting to 1/20th of the single large dose, the excretion figures are far higher than with the single small dose, and fall very slightly short of the figures for the single large dose. The percentage mortality is, however, no higher than with a single small dose, and very definitely less than with a single large dose. The effect of repeated dosage, *per os*, with mouse-typhoid bacilli has recently been studied by Lange (1924), with results of the greatest interest. Our results, which

afford no evidence of an increased mortality with repeated dosage, appear at first sight to be at variance with those which Lange records. It is, however, quite probable that the contradiction is no more than apparent. The size of the doses administered, and still more perhaps the number of doses and the intervals between them, are probably of decisive importance. In any case the issues raised by Lange's results are of such importance, that it seems wiser to postpone any further discussion of this question until further data are available.

SUBACUTE, CHRONIC AND LATENT INFECTIONS.

We have commented, in several previous reports, on the frequency of subacute, chronic, and latent infections among experimentally infected mice, especially as evidenced by the isolation of *B. aertrycke* from the spleens of animals which have survived for more than 42 days after infection. The extensive series of mice studied in our previous investigation enabled us to give figures showing the frequency of this type of infection in relation to the dose of bacteria administered (Topley and Ayrton, 1924 *b*). The series of experiments here reported has added considerably to the data available for the study of this condition. The results are summarised in Table XXII, and it will be noted that they entirely confirm those already recorded.

Table XXII.

Showing the proportion of mice, surviving beyond a definite period after inoculation or feeding with cultures of B. aertrycke, *which yielded cultures of that organism from their spleens.*

Series	History of mice	No. of mice	No. with positive spleen cultures	Percentage with positive spleen cultures
1	Survived for 21 days after intraperitoneal or subcutaneous inoculation	28	24	85·7
2	Survived for 42 days after receiving one large dose of *B. aertrycke* per os	98	60	61·3
3	Survived for 42 days after receiving one small dose of *B. aertrycke* per os	39	13	33·3
4	Fed on 4 small doses of *B. aertrycke* and survived 42 days after date of first feeding	32	16	50

We must conclude that latent infection is a frequent result of the administration of sublethal doses of *B. aertrycke* to mice. The condition would appear to involve a well-established equilibrium between parasite and host, for the mice show no evidence of ill-health during life, and when killed they show, in most cases, no recognisable lesions at autopsy. There may be some slight splenic enlargement, but in the great majority of cases the size of the spleen lies within the limits which we must regard as normal for the mouse. The growth of *B. aertrycke*, when a portion of the spleen tissue is incubated in nutrient broth, is the only evidence that any form of infection exists.

THE VIRULENCE OF STRAINS OF *B. AERTRYCKE* ISOLATED FROM SURVIVORS.

It is of some interest to determine the minimal lethal dose of cultures of *B. aertrycke* derived from the spleen tissue of these surviving mice. It would afford some explanation of the tolerance of the mouse tissues for the bacteria which they harbour, if we could demonstrate that these had become so modified as to be less virulent than the strains originally administered. It has indeed frequently been suggested that some such change does occur in bacteria which have passed into the tissues, and there vegetated. Many workers have reported experiments bearing on this point. So far as our own results are concerned we have obtained no evidence pointing in this direction.

We have tested strains of *B. aertrycke* isolated from the spleen of mice, which had survived for 42 days after infection, had shown no sign of ill-health, and had presented no lesions indicative of infection at autopsy. In order that the opportunities for reversion to a virulent type in artificial culture should be reduced to a minimum, we employed for inoculation the primary broth cultures, containing the portions of spleen tissue, after 18 hours' incubation at 37° C. The fact that the cultures contained *B. aertrycke* was determined by the preliminary withdrawal of a small sample of the culture fluid, which was tested by agglutination. The purity of the cultures was confirmed by subsequent plating. The results are shown in Table XXIII.

Table XXIII.

Showing results obtained by inoculating mice intraperitoneally with direct broth cultures from the spleens of mice which had survived for 42 days after feeding with B. aertrycke.

	Strain							
	T 5		G 5		T 6		G 6	
Dose	Day		Day		Day		Day	
0·25	1	1*	1	1	1	1	1	1
0·025	1	4	2	1	2	2	2	2
0·0025	4	6	6	1	8	5	5	6
0·00025	3	4	4	12	4	12	4	8
0·000025	5	5	6	6	S	S	S	10

* Two mice were inoculated with each dose. The figures given indicate the day after inoculation on which each mouse died. In every case the mouse presented typical lesions and *B. aertrycke* was isolated from the tissues.

S = survived for 21 days.

These strains are clearly possessed of the normal degree of virulence, as tested by intraperitoneal inoculation. The equilibrium arrived at in the splenic tissue does not appear to be associated with any loss of virulence on the part of the parasite, once that parasite has commenced to multiply freely in a new environment.

THE PRESENCE OF AGGLUTININS IN THE BLOOD OF SURVIVING MICE.

In only one series of animals was this point examined. Of 68 mice which had survived for 42 days after being fed on a single large dose of *B. aertrycke*, six showed the presence of agglutinins in the blood serum. The titres obtained varied between 1/40 and 1/1280. The sera from the remaining 62 mice showed no reaction in a dilution of 1/20. All six mice with agglutinins in the serum gave cultures of *B. aertrycke* from the spleen. Of the 62 mice showing no agglutinins, 34 gave positive, and 28 negative spleen cultures.

We have seen that an equilibrium may be established between parasite and host, in virtue of which the bacteria may continue to subsist in the splenic tissues. We have failed to find any evidence that this equilibrium is dependent on a decrease of bacterial virulence: we have equally failed to demonstrate the presence of agglutinins in the great majority of those mice in which this equilibrium had been established, and, to this extent, we have failed to find evidence of an active immunity of the humoral type.

It must, however, be recognised that the absence of agglutinins does not exclude the presence of other anti-bodies. Wolf (1908) failed to demonstrate agglutinins in the serum of mice immunised by living cultures of mouse-typhoid bacilli, administered *per os*, but was able to show that antibodies were present which gave rise to the Pfeiffer phenomenon when mixtures of mouse-serum and bacterial suspension were inoculated intraperitoneally into guinea-pigs.

SUMMARY.

We may summarise our results, including those recorded in our two recent reports (Topley and Ayrton, 1924 *b* and *c*) under the following headings.

(1) *The antigenic structure of* B. aertrycke (*Mutton*).

(*a*) *B. aertrycke* (Mutton) undergoes natural dissociation into two sharply distinguished varieties, one containing that antigenic constituent which is characteristic of this bacterial species or variety, the other that antigenic constituent which *B. aertrycke* (Mutton) shares with the bacterial group to which it belongs. We have obtained no evidence that both the type and group antigen may be fully developed in one and the same bacillus. In these respects our results confirm entirely those recorded by Andrewes (1922).

(*b*) Both the type and the group varieties of this organism, when grown for more than 16 hours at 22° C., or for that length of time at 37° C., tend to undergo an antigenic alteration such that they become agglutinable with an entirely different type of antibody, to which they did not previously respond. The antigen developed as a result of this alteration would seem to be the same for the type and the group varieties. Since plating from such altered cultures gives colonies of the usual type or group variety, it would seem probable that the altered bacilli are dead, dying or senescent organisms.

(*c*) Although type or group cultures readily give rise to individuals of the alternative antigenic variety in ordinary laboratory media, yet this change is

neither so rapid nor so unpredictable in its occurrence that it is impossible to determine the nature of any given colony, or to obtain a culture of the serological variety required.

(*d*) There is some evidence that type variants arise in group cultures more readily than do group variants in type cultures.

(2) *The relations between the presence of type or group antigen and other biological attributes of* B. aertrycke.

(*a*) Roughness and smoothness vary independently of the presence of group or type antigen. Rough forms may react as group or type.

(*b*) There is no difference between the virulence of the type and group varieties of *B. aertrycke*. While the virulence of all smooth strains is high, the virulence of all rough strains is low.

(*c*) There is some evidence that group strains of *B. aertrycke* give rise more readily to faecal excretion than do type strains, but the difference is not great. Rough strains appear, in this as in other respects, to be ill fitted for a parasitic existence, and do not give rise to persistent faecal excretion.

(*d*) There is a well-marked tendency for group strains, which have gained access to the tissues, to give place to the type variety. The longer the sojourn in the tissues, the more complete is this replacement. Type strains, under similar conditions, do not tend to be replaced by group strains.

There is a similar, but less marked, tendency for group strains to be replaced by type strains in the intestinal canal.

(3) *The excretion of* B. aertrycke *in the faeces, after administration by the mouth, and its relation to other phenomena of infection.*

(*a*) The excretion of *B. aertrycke* in the faeces, after administration *per os*, may be continuous or intermittent, or may not occur in a sufficient degree to be detected by the technique employed.

(*b*) The faecal excretion, in those mice which succumb to infection, is no more regular in its course than in those mice which survive. It is a frequent occurrence for mice to die with typical lesions of enteric infection, without ever having excreted *B. aertrycke* in detectable amounts.

(4) *The condition of mice which have survived the oral administration of* B. aertrycke.

(*a*) A high proportion of mice, which have survived for 42 days after the oral administration of *B. aertrycke*, yield cultures of this organism from their spleens when examined post-mortem. In many series of experiments the proportion of survivors showing such positive spleen cultures has exceeded 50 per cent.

(*b*) The strains of *B. aertrycke* isolated from the spleens of such survivors appear to be possessed of the normal degree of virulence.

(*c*) The presence of agglutinins in the blood serum can be demonstrated in only a very small proportion of such surviving mice.

(5) *The relation of dosage to the phenomena studied.*

(*a*) With a single dose, there is a definite relation between the number of viable *B. aertrycke* administered and the frequency of the phenomena indicating infection. The larger the dose the higher is the mortality rate, the more frequent and persistent is the faecal excretion, and the higher is the percentage of the survivors which harbour *B. aertrycke* in their tissues.

As the dose decreases there is, at first, a rapid fall in the frequency of these various phenomena, but when the dose falls below a certain limit further decreases produce relatively little effect, over the range of doses studied.

(*b*) Repeated administration of small doses of *B. aertrycke* results in a high frequency of persistent faecal excretion.

In conclusion we should wish to express our thanks to Miss E. R. Lewis, for her assistance during a large part of the investigations recorded above.

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