THE EXCRETION OF *B. ENTERITIDIS* (*AERTRYCKE*) IN THE FAECES OF MICE AFTER ADMINISTRATION BY MOUTH.

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(With 10 Charts and 1 Text-figure.)

In the preceding report (Topley and Ayrton, 1923 *a*) we have described a technique by which measurements may be obtained of the excretion of *B. enteritidis* (*aertrycke*)[1] in the faeces of mice.

For reasons which have been set out in the report referred to above, we have adopted an arbitrary method of scoring the number of viable *B. aertrycke* present in any given specimen of faeces, using a series of class-intervals which increase in extent in a geometric ratio, and assigning the same score to all results which fall within the same class-interval.

In the present report we describe the results of a considerable series of experiments in which we employed this technique in the endeavour to obtain an answer to the two following questions:

(1) Do different strains of *B. aertrycke* vary in the manner in which they are excreted in the faeces of mice, following administration by the mouth?

(2) What, if any, is the relation between the dose of viable bacilli administered, and the frequency, amount or persistence of the subsequent faecal excretion?

The results obtained in studying the first of these two questions were so striking, that the investigation has largely concerned itself with the points raised in this part of the work. The question of dosage has, however, never been lost sight of, and all the available data are considered in the latter part of this report.

GENERAL TECHNIQUE.

The technique which has been adopted in the experiments under consideration is as follows:

In the majority of cases one feeding only of bacterial culture is administered to each batch of mice. In each experiment ten mice are fed. A preliminary examination of the faeces of each mouse is carried out to determine the absence of *B. aertrycke*. To each mouse we then administer 0·1 c.c. of an

[1] The organism referred to is of the "Mutton" variety. For convenience it will in future be referred to simply as *B. aertrycke*.

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18 hours' broth culture of *B. aertrycke*, or a dilution of it in nutrient broth, by allowing five drops of the fluid to fall into the opened mouth of the mouse from a dropping pipette, calibrated to deliver 50 drops per c.c. An interval of a few minutes is allowed to elapse between giving the first three drops and the last two, to render swallowing easier and more certain. This method is more accurate than administration by feeding on bread soaked in bacterial culture, and it appears to us that it must give a more exact representation of the course of events in the natural spread of infection than is obtained by intra-stomachal injection through a catheter, as in the method employed by Webster (1922).

To each mouse, in each experiment, we have given five drops of culture. In the case of the first pair, in each batch of ten, the culture is undiluted: in the case of the second pair it is diluted ten times: in the case of the third pair one hundred times, and so on. In each series, therefore, the actual doses administered are as follows:

Mice	1	and	2	...	0·1 c.c. of an 18 hours' broth culture		
,,	3	,,	4	...	0·01	,,	,,
,,	5	,,	6	...	0·001	,,	,,
,,	7	,,	8	...	0·0001	,,	,,
,,	9	,,	10	...	0·00001	,,	,,

In all experiments each mouse is kept throughout in a separate cage, so that there is no opportunity for passage of bacilli from mouse to mouse. Each batch of mice is observed for 42 days, the faeces being examined on the second and third days after feeding, three times during each of the following two weeks, twice during each of the next three weeks, and on the 41st and 42nd days. In some cases more frequent examinations have been made.

Any mouse which dies is examined post-mortem, according to the routine procedure which has already been described (Topley, 1922). On the 42nd day all surviving mice are killed, with a few exceptions in which an individual mouse is retained for some special reason. A post-mortem examination is made, and any abnormality noted. A portion of the spleen is excised and dropped into a tube of broth, which is incubated for two days at 37° C. before being discarded as sterile. If any growth occurs, sub-cultures are made on plates of McConkey's medium.

In all cases in which colonies are obtained, whether from faeces, from the tissues of mice dying from enteric infection, or from the spleen cultures from survivors, which on general grounds are regarded as probably *B. aertrycke*, such colonies are sub-cultured to broth, killed by the addition of formalin followed by heat, and tested against high-titre agglutinating sera, as will be referred to in more detail later.

In the case of each mouse, therefore, we may find evidence of infection, using this term in its broadest sense, in three distinct ways. We may isolate *B. aertrycke* from the faeces: the mouse may die of *B. aertrycke* infection or, dying from some intercurrent disease may yield cultures of *B. aertrycke* from

its tissues: or it may remain in apparent health until it is killed after 42 days, but from its spleen, removed after death, *B. aertrycke* may be isolated.

It will be convenient, as regards the question of bacterial variation, to state at once the general conclusions arrived at, and then to produce our evidence, describing a few experiments in detail and finally summarising the results of the whole series.

Our results would seem to show that:

(*a*) Wide variations occur in the behaviour of different strains of *B. aertrycke*, as judged by the excretion of this organism in the faeces of mice, after it has been administered by the mouth.

(*b*) These variations are discontinuous. Certain strains are excreted in the faeces of a high proportion of the mice to which they are fed, and this excretion may persist over considerable periods of time. Other strains are either not excreted at all, or they are excreted by few of the mice to which they are fed, and then the excretion is transient. The feature which distinguishes sharply between the two kinds of strain is the capacity for giving rise to persistent excretion. Where such capacity is demonstrated the strain in question always belongs to a given variety, which may be recognised in other ways. Where such a capacity cannot be demonstrated, in so limited a series of mice as the ten employed in each of these experiments, it is not safe to assume that the strain in question does not belong to this variety, although the probability is strongly against its doing so. The contrasted variety never gives rise to persistent excretion, so far as can be recognised by the technique employed.

(*c*) The strains which are persistently excreted are those which are agglutinated with a "group" anti-serum, using this term in the sense attached to it by Andrewes (1922). They may also react to a "type" or monospecific antiserum. It follows that they always contain group antigen, and may or may not contain type antigen.

(*d*) The strains which are not so excreted are those in which group antigen is absent. They contain the type antigen alone.

(*e*) These results hold true both for smooth and for rough varieties of *B. aertrycke*; but it is much less common to get persistent excretion of a rough than of a smooth strain. Where persistent excretion of a rough strain does occur, the strain always possesses group antigen.

It will be well first to consider, very briefly, the relevant facts as regards the serological relationships of *B. aertrycke*. It will suffice, for the moment, to recall the crucial results obtained by Andrewes (1922).

Following a line of reasoning which is fully set out in the paper referred to, Andrewes made use of two kinds of agglutinating sera. One of these was strictly specific. It was prepared by thoroughly absorbing a high-titre aertrycke serum with closely related organisms, such as *B. paratyphosus* B which were themselves agglutinated by this serum in its untreated state. The other was a paratyphosus B serum which *ex hypothesi* did not contain the specific or type

agglutinin, but which was known to give group agglutination with *B. aertrycke* in high dilution.

In this way, Andrewes was armed with one reagent which would pick out type antigen, and another which would pick out group antigen. He found, when numerous colonies of a given strain of *B. aertrycke* were subcultured into broth and these broth cultures were killed by the addition of formalin and tested against these monospecific and group sera, that the strains so isolated fell sharply into two classes, the one agglutinating to titre, or almost to titre, with the type serum, and failing to react with the group serum, the other behaving in exactly the opposite way. With some strains, however, there was slight cross agglutination, while very occasionally Andrewes met with strains which agglutinated well with both sera. The striking fact stood out clearly, that from any given culture of *B. aertrycke* two kinds of strain could be separated, one reacting with type antiserum, the other with group antiserum.

It was, however, evident in all Andrewes' work that these type and group varieties were markedly unstable in culture. One variety might change into the other with startling and disconcerting rapidity. There was, it should be emphasised, no question of a variation which would transgress the limits recognised as demarcating one species or type from another. *B. aertrycke* never changed into *B. paratyphosus* B, nor *vice versa*. It was impossible to differentiate a group strain of *B. aertrycke* from a group strain of *B. paratyphosus* B by agglutination, but when either of these group strains gave rise to type variants, these were always *B. aertrycke* or *B. paratyphosus* B as the case might be. Thus it seemed clear that the group variety of *B. aertrycke*, for instance, always possessed a minimal amount of type antigen. There was, at least, some controlling factor which ensured that, when the type antigen did appear in detectable amounts, it was always of the aertrycke type and never of any other.

We may say at once that our work has confirmed the results obtained by Andrewes in all important respects. There are two slight exceptions. We have met, far more frequently than he appears to have done, strains of *B. aertrycke* which react equally well with type and group antisera. We consider these more fully in a further report (Topley and Ayrton, 1924[1]) and for the moment we would merely state that we should be disposed to recognise at least three varieties of *B. aertrycke*, (*a*) type strains, (*b*) group strains, and (*c*) mixed strains.

We have on several occasions met with strains which appear to have lost all agglutinability. These latter have played no part in the present series of experiments, and we shall not, for the moment, consider them further.

As mentioned in the preceding report, in every case in which brown colonies were encountered in making a bacterial count on a specimen of faeces, indicating the formation of hydrogen sulphide, a selection of these colonies were subcultured to broth and tested against high-titre agglutinating

[1] To appear in *Journal of Hygiene*, XXII. No. 3.

sera. In all but the first four of the experiments here reported the sera employed were (*a*) a high-titre mono-specific (absorbed) aertrycke serum, (*b*) a high-titre newport serum, known to agglutinate group strains of *B. aertrycke*, and (*c*) a high-titre gaertner serum, also known to agglutinate some group strains of *B. aertrycke*.

In this way it was possible to divide all strains isolated from the faeces into type, group or mixed strains, on the basis of the agglutination results. All strains isolated from the tissues were tested in an identical manner.

In the first four experiments, the aertrycke serum employed had not been absorbed. In these experiments, therefore, we are only justified in dividing the strains into two classes: (*a*) type strains, and (*b*) group or mixed strains. Due allowance is made for this in all tables, etc.

VARIABILITY OF STRAINS IN CULTURE.

The thesis which we consider to be supported by our results relates a particular type of agglutination reaction to a particular type of behaviour as regards excretion. It is clear that, for this argument to be valid, we must place a reasonable degree of reliance on the results of our agglutination tests, in the sense that we can judge from them the kind of organisms that were present in the faeces.

In this connection we would cite the following facts.

It is no unusual event to isolate, from the faeces of a given mouse, a series of strains of *B. aertrycke* of one serological type, and one only. For instance, from the faeces of five mice in one experiment, 58 strains of *B. aertrycke* were isolated. All agglutinated to titre with a group antiserum, but none gave any agglutination with a type antiserum. Results of this kind have been of frequent occurrence. Indeed it has been very unusual to isolate pure group and pure type varieties of *B. aertrycke* from the same mouse or series of mice, except in a few cases where mixed cultures have purposely been administered. We may also note the fact that approximately 93 per cent. of all strains of *B. aertrycke* isolated from the faeces have reacted with a group antiserum. Such results could not be the consequence of spontaneous variations in sub-culture, if such variations proceeded with equal readiness in either direction. They could only be explained, on such a basis, by supposing that the change from type to group occurred very frequently, while the change in the opposite direction occurred very rarely.

If such were the case, we should not expect, in any series of experiments, to obtain a preponderance of strains reacting with the type antigen alone. In three experiments, in which the excretion of *B. aertrycke* was very scanty, we isolated 54 strains of this organism from the faeces. Fifty-one of these strains, or 94 per cent., agglutinated with a type antiserum but not with a group antiserum.

Finally, we would cite certain experiments, referred to in a later report (Topley and Ayrton[1]), in which we inoculated cultures of *B. aertrycke*

[1] See footnote, p. 237.

intraperitoneally into mice, and tested by agglutination numerous strains recovered from the tissues after death. Two series, each of ten mice, were inoculated with strains believed to be of the pure type variety. From the tissues of the mice which died, or were killed, 382 strains of *B. aertrycke* were recovered. Of these, 330 agglutinated with a type serum only, 8 agglutinated with a group serum only, and 44 agglutinated with both sera. Two other series of ten mice each were inoculated with strains believed to be of the pure group variety. From the tissues of mice which died or were killed 313 strains of *B. aertrycke* were isolated. Of these, 231 agglutinated with a group serum only, one agglutinated with a type serum only, and 81 agglutinated with both sera.

It seems probable that the spontaneous variation, which undoubtedly occurs in broth cultures, is not of such a nature as seriously to obscure the true state of affairs, with regard to the distribution of type and group varieties in the tissues or excretions from which the cultures were obtained.

Turning now to the question of our control over the cultures which we administer by mouth, the position is much less satisfactory. We can determine the nature of such cultures only by testing a sample at the shortest possible interval before administration, and here we encounter a formidable difficulty.

We desire to know the nature of the viable bacilli in the culture we are administering, but our test sample will tell us only the distribution of the two kinds of antigen among all bacilli in the culture, the living and the dead. It is probable that, if a relatively small proportion of all the bacilli were of a different variety from that we desire to administer, we should still detect them by agglutination against high-titre sera. It is easy to show, however, that this does not apply when we consider the viable bacilli alone.

A flask of broth was inoculated from a culture of *B. aertrycke*, which we believed to contain only the type variety, and was then incubated at 25° C. After various intervals a sample of the culture was withdrawn, killed with formalin and heat, and agglutinated. At the same time a plate culture was prepared. Next day 50 colonies were picked from this plate, subcultured into broth, grown for 18 hours, at 25° C., killed and agglutinated. Thus we had a record of the agglutination reaction of the original culture after various periods of incubation, while the results obtained with the colonies from the plates gave us a picture of the nature of the viable portion of the bacillary population at the same moment.

Without giving detailed results, it is sufficient to record that after six hours the original culture agglutinated with a type serum alone, while from a plate prepared at this time we isolated 43 colonies of type bacilli and 7 mixed. On another occasion, when the original culture still responded only to the type antiserum, 17 of the 50 colonies subcultured from the corresponding plate reacted with both type and group antisera.

EXPERIMENTAL RESULTS.

The striking difference between the quantitative excretion of *B. aertrycke*, which followed the administration to mice of different strains of this organism, was noted very early in the course of our investigation. The routine examination of a selection of all strains, by testing them against the three agglutinating sera, at once brought to our notice the fact that the great majority of these strains were agglutinated by the group serum, and that strains which were agglutinated by the type serum alone were very rarely isolated from the faeces. Past experience had shown us that such strains were quite common in cultures obtained from the tissues during epidemics of enteric infection in mice.

In all succeeding experiments, therefore, an attempt was made to administer a strain of known serological variety. Colonies were picked from a plate culture, obtained from a growth in broth which had itself responded to agglutination by showing purely type or purely group affinities. Six to twelve of these colonies were subcultured to broth tubes containing 10 c.c. of medium. These tubes were incubated aerobically at 22–25° C., or anaerobically at 37° C. Very numerous tests had shown us that, under either of these conditions, there was less probability of spontaneous variation than if the cultures were incubated aerobically at 37° C. As the result of a limited number of experiments we were led to the view that anaerobic incubation had a very definite effect in retarding these variations, but more extended experience has led us to doubt whether anaerobiosis acts in any other way than in slowing the rate of bacterial multiplication, though it has proved to be one of the most certain methods of inhibiting a rapid change from one serological variety to another.

After 16 to 17 hours' incubation the tubes were removed from the incubator, a sample was taken from each of them, killed by formalin and heat and tested against the three sera employed throughout the investigation. Any culture which gave indication of possessing both type and group antigen was discarded, and one of the cultures which were sharply differentiated as belonging to the variety desired was employed for feeding purposes.

Other technical details, concerning methods of administration, dosage, etc., have been referred to above and need not be repeated. The methods employed in recording and charting the results may however be briefly recapitulated.

The actual bacterial counts are first expressed as numbers of viable *B. aertrycke* per c.c. of a faecal suspension of a turbidity corresponding to a bacterial content of 1,000,000,000 per c.c. Any count not greater than 10 is scored as 1, any count greater than 10 but not greater than 100 is scored as 2 and so on, using in all cases the index or logarithm of the first integral power of 10 greater than the actual count recorded. The reasons for adopting this convention have been discussed in a previous report (Topley and Ayrton, 1923[a]).

In the charts, the results for each mouse are recorded along a separate base-line. A curve of excretion is drawn, using the figures scored for the bacterial counts in the manner described above. The area between this curve and the base-line is blackened to increase the clearness of representation. A time-scale is placed below the whole series of curves, and also on an upper base-line. Along each base-line, corresponding to an individual mouse, are recorded the days on which bacterial counts of the faeces were carried out. The record of each individual mouse terminates in an arrow pointing upwards. Where these arrows indicate deaths occurring during the course of the experiment, a corresponding square will be found on the upper base-line. This is black if a post-mortem examination proved the death to be due to enteric infection. It is unshaded if no evidence of such infection could be obtained. On the last day of each experiment, those mice which remained alive were killed, and each mouse is represented by an upward pointing arrow with a circle at its lower extremity. A post-mortem examination was carried out on each mouse, and cultures were prepared from the spleen. If any given spleen culture yielded a growth of *B. aertrycke*, the circle beneath the corresponding arrow is blackened. If the spleen culture proved negative the circle is left unshaded.

One other figure, which is entered in the tables and in the records of those experiments which are referred to in more detail, needs an explanation. It is recorded as the "excretion coefficient." It was clearly desirable to adopt some numerical expression as a measure of the total excretion during any given experiment of any mouse, or group of mice. One very simple method of arriving at such an expression is to record the percentage of all specimens of faeces examined from such a group, which gave positive results. We are not, at the present time, prepared to maintain that this measure is in any way inferior to the one we have adopted. So far as the present enquiry is concerned it gives almost the same relative results. It does not, however, take into account the number of bacilli excreted. It does not differentiate between a specimen of faeces containing viable *B. aertrycke* in such numbers as to give an almost pure culture, and another specimen in which they form a tiny minority of the viable bacteria present.

We have therefore adopted a formula which takes account of the relative number of viable *B. aertrycke* in the positive specimens. In arriving at the excretion coefficient for any group of mice, we have added together the scores obtained by all specimens of faeces examined, divided the sum by the total number of specimens examined and multiplied the resulting figure by 100. The coefficient thus expresses the total score which would be obtained in examining 100 specimens of faeces, assuming that the distribution of *B. aertrycke* was the same as that actually observed.

The following experiments will serve as examples of the whole series. We have simply recorded the results obtained without textual description.

Exp. D. Chart I. Ten mice were fed on an 18 hours' broth culture of *B. aertrycke* (smooth group or mixed strain) and observed over 42 days with the following results:

Number of mice fed	10
Number of mice which excreted *B. aertrycke*	8
Number of specimens of faeces examined	138
Number of specimens of faeces positive	31
Excretion coefficient	74
Number of colonies from faeces tested by agglutination	168
Number of colonies reacting as Type	0
Number of colonies reacting as Mixed or Group	168
Number of mice which died	4
Number of mice positive on post-mortem examination	3
Number of colonies from the tissues of these mice tested by agglutination	18
Number of colonies reacting as Type	0
Number of colonies reacting as Mixed or Group	18
Number of survivors killed on 42nd day	6
Number of survivors with positive spleen cultures	3
Number of colonies from these cultures tested by agglutination	9
Number of colonies reacting as Type	0
Number of colonies reacting as Mixed or Group	9

The agglutinating serum used in this experiment did not allow us to differentiate between group and mixed strains. We would call attention to the high proportion of mice excreting *B. aertrycke*, the persistence of the excretion in certain cases, and the fact that all strains isolated contained group antigen. It may also be noted that the examination of those mice which survived, in apparently perfect health, until the 42nd day showed that half of them were harbouring viable *B. aertrycke* in their spleen tissue.

Exp. E. Chart II. Ten mice were fed on an 18 hours' broth culture of *B. aertrycke* (smooth type strain) and observed for 42 days with the following results:

Number of mice fed	10
Number of mice which excreted *B. aertrycke*	0
Number of specimens of faeces examined	139
Number of specimens of faeces positive	0
Excretion coefficient	0
Number of mice which died	2
Number of mice positive on post-mortem examination	1
Number of colonies from the tissues of this mouse tested by agglutination	20
Number of colonies reacting as Type	14
Number of colonies reacting as Group	0
Number of colonies reacting as Mixed	6
Number of survivors killed on 42nd day	8
Number of survivors with positive spleen cultures	2
Number of colonies from these cultures tested by agglutination	20
Number of colonies reacting as Type	7
Number of colonies reacting as Group	0
Number of colonies reacting as Mixed	13

In this experiment the feeding of a pure type culture was followed by the entire absence of faecal excretion; but at least three mice became infected, since one mouse died of typical enteric infection and two mice were found to be harbouring *B. aertrycke* in their spleen tissue after 42 days.

It may be noted that mixed as well as type cultures were isolated from the tissues, yet no mixed strains ever appeared in the faeces.

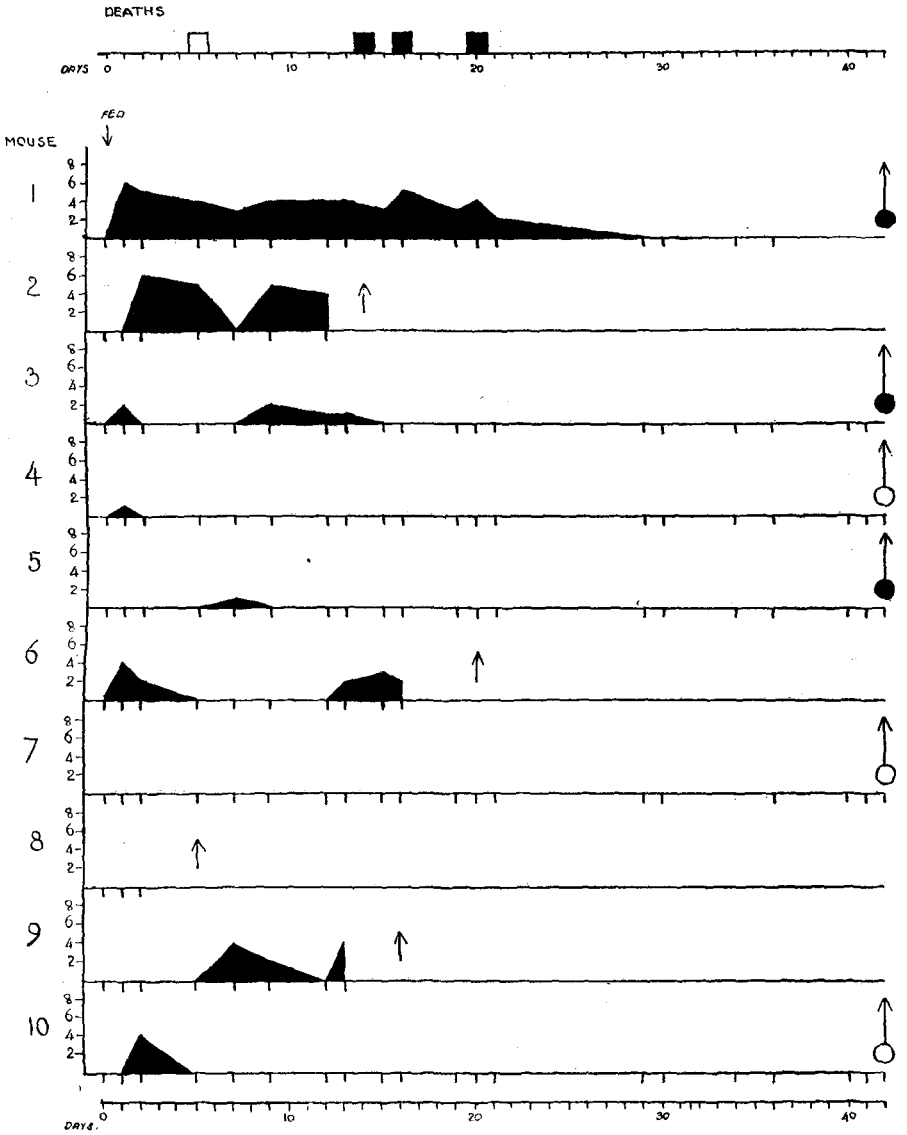

Chart I. Exp. *D.*

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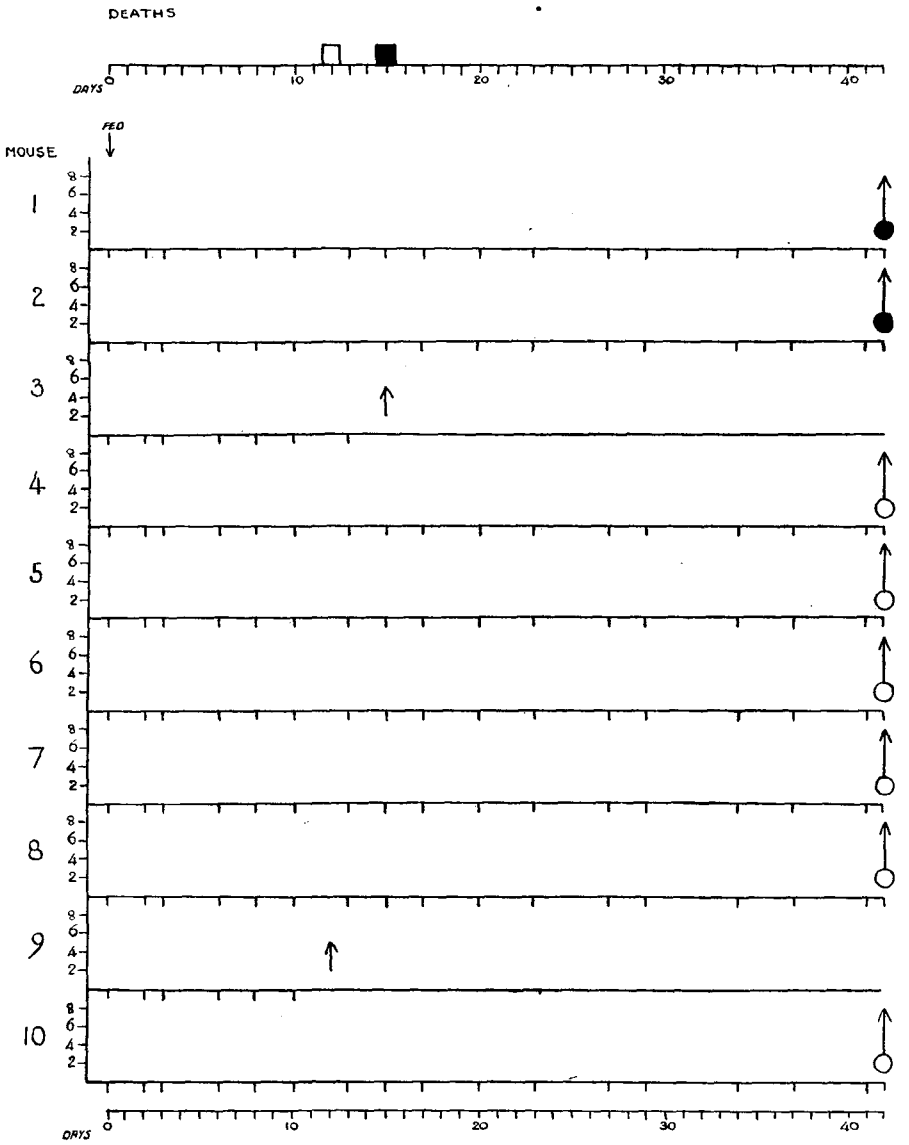

Chart II. Exp. *E.*

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Exp. F. Chart III. Ten mice were fed on an 18 hours' broth culture of *B. aertrycke* (smooth type strain) and observed for 42 days with the following results:

Number of mice fed	10
Number of mice which excreted *B. aertrycke*	3
Number of specimens of faeces examined	144
Number of specimens of faeces positive	5
Excretion coefficient	13
Number of colonies from faeces tested by agglutination	15
Number of colonies reacting as Type	15
Number of colonies reacting as Group	0
Number of colonies reacting as Mixed	0
Number of mice which died	2
Number of mice positive on post-mortem examination	2
Number of colonies from the tissues of these mice tested by agglutination	39
Number of colonies reacting as Type	27
Number of colonies reacting as Group	0
Number of colonies reacting as Mixed	12
Number of mice killed on 42nd day	8
Number of mice with positive spleen cultures	0

In this experiment there was transient excretion on the part of three mice, and two mice died of typical enteric infection.

Exp. A. Chart IV. This is an example of the failure of an attempt to feed ten mice on a pure type strain. The mice were observed for 42 days with the following results:

Number of mice fed	10
Number of mice which excreted *B. aertrycke*	6
Number of specimens of faeces examined	143
Number of specimens of faeces positive	56
Excretion coefficient	129
Number of colonies from faeces tested by agglutination	286
Number of colonies reacting as Type	0
Number of colonies reacting as Mixed or Group	286
Number of mice which died	3
Number of mice positive on post-mortem examination	2
Number of colonies from the tissues of these mice tested by agglutination	12
Number of colonies reacting as Type	0
Number of colonies reacting as Mixed or Group	12
Number of survivors killed on 42nd day	3
Number of survivors with positive spleen cultures	0

Four mice from this experiment were saved alive, in order to test the length of time over which excretion would continue.

This is one of the four experiments in which the sera employed did not differentiate between group and mixed strains. It is of interest because, although we failed to exclude group or mixed bacilli from the culture used for feeding, there can be no reasonable doubt that we administered viable type bacilli. It therefore illustrates the point that a culture containing both type and group bacilli tends to behave, as regards excretion in the faeces, in the same way as a group culture containing group or mixed bacilli only. Thus, even when an experiment in which an attempt to feed a group strain appears to have been entirely successful, we are not justified in assuming that we have administered no type bacilli.

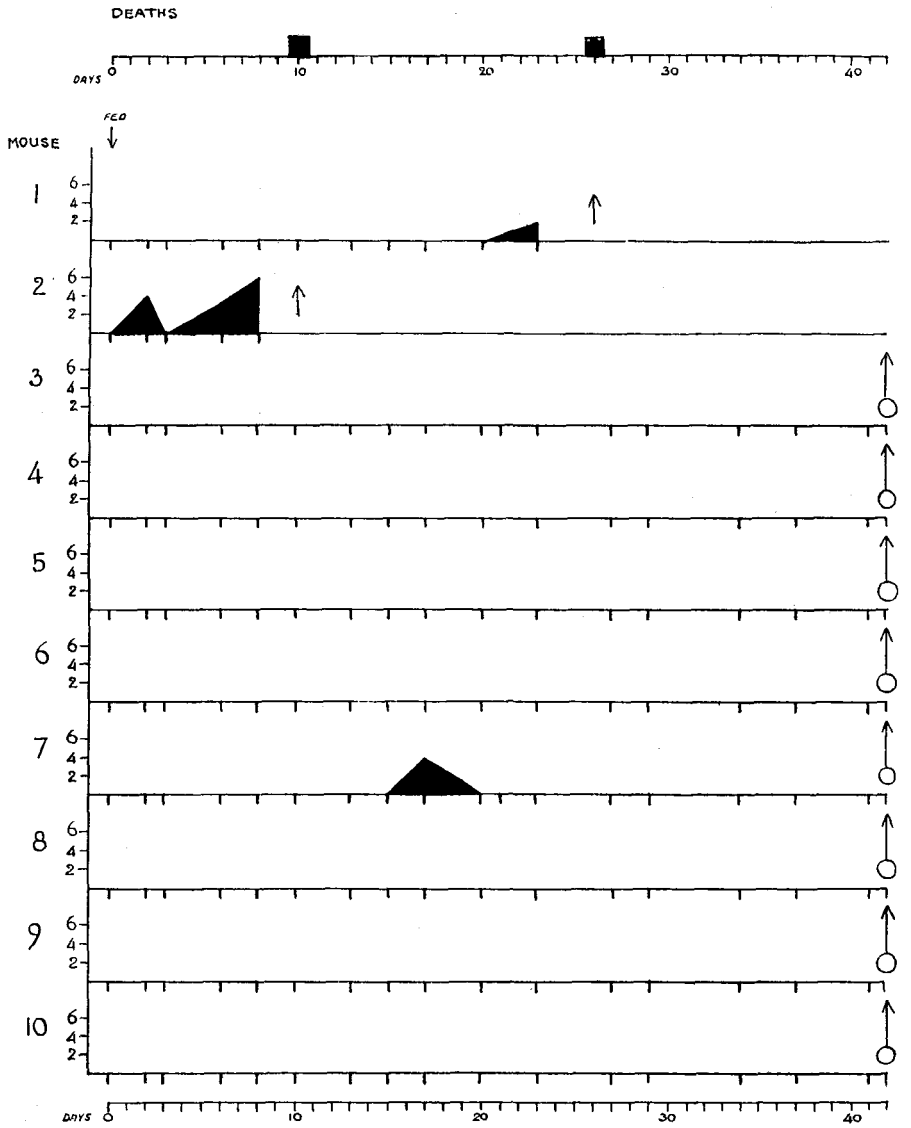

Chart III. Exp. *F.*

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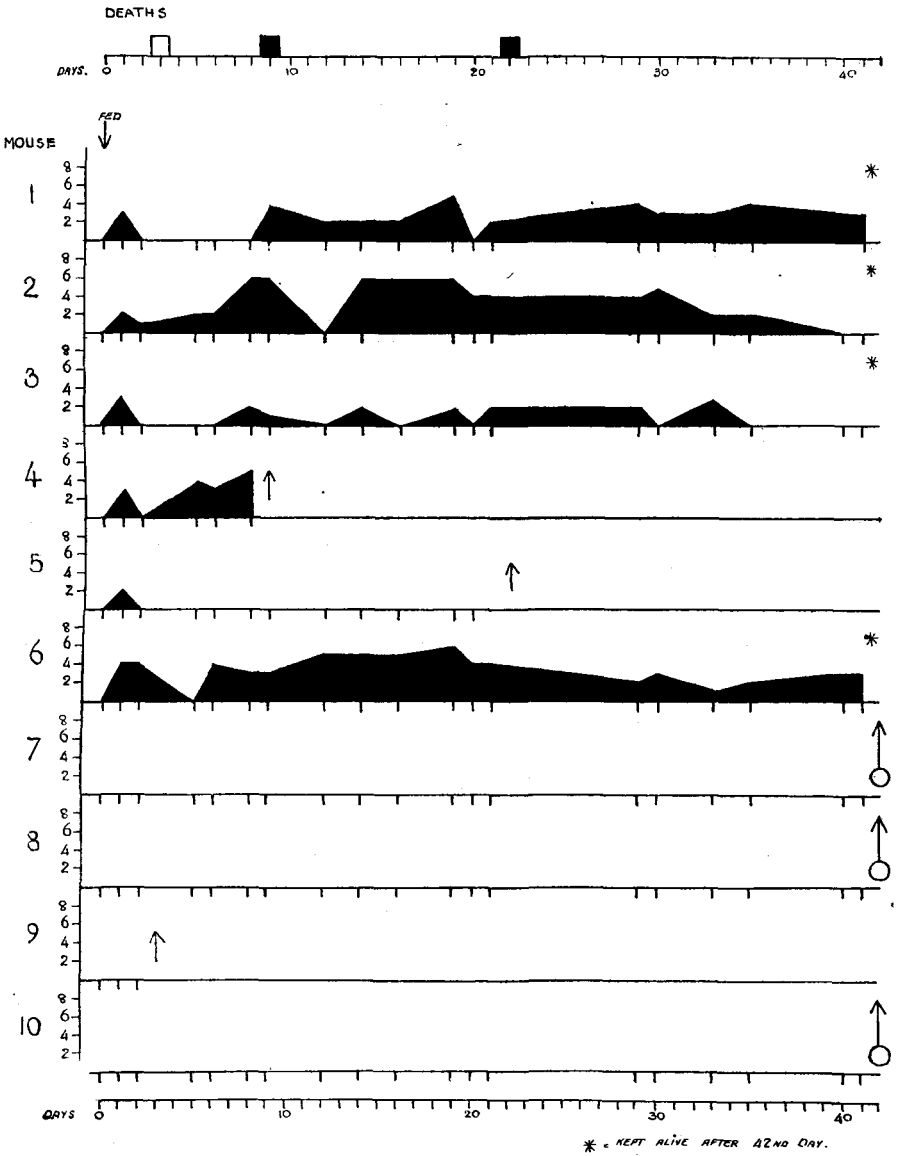

Chart IV. Exp. *A*.

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Exp. J. Chart V. Ten mice were fed on a mixture containing equal parts of two cultures of *B. aertrycke*, one a smooth type strain, the other a smooth group strain. The mice were observed for 42 days with the following results:

Number of mice fed	10
Number of mice which excreted *B. aertrycke*	3
Number of specimens of faeces examined	137
Number of specimens of faeces positive	19
Excretion coefficient	49
Number of colonies from faeces tested by agglutination	76
Number of colonies reacting as Type	8
Number of colonies reacting as Group	55
Number of colonies reacting as Mixed	13
Number of mice which died	2
Number of mice positive on post-mortem examination	2
Number of colonies from the tissues of these mice tested by agglutination	40
Number of colonies reacting as Type	20
Number of colonies reacting as Group	16
Number of colonies reacting as Mixed	4
Number of survivors killed on 42nd day	8
Number of survivors with positive spleen cultures	3
Number of colonies from these cultures tested by agglutination	30
Number of colonies reacting as Type	0
Number of colonies reacting as Group	2
Number of colonies reacting as Mixed	28

As in the last experiment, group or mixed bacilli dominate the situation as regards faecal excretion, but it will be noted that the distribution of type and group strains in the tissues differs from that in the faeces.

All the experiments so far recorded were carried out with smooth strains. In view of the knowledge which we already possess, with regard to the differences in the behaviour of smooth and rough strains when introduced into the tissues, it was natural that we should investigate the behaviour of rough variants when administered by the mouth. The general relation of roughness and smoothness to the other factors investigated is discussed in another report (Topley and Ayrton, 1924[1]), but we include the feeding experiments in this paper for the sake of completeness.

The rough variants were obtained by allowing broth cultures of *B. aertrycke* to remain in the incubator at 37° C. for 7 to 10 days or longer, then plating and picking off the rough colonies, which seldom failed to appear in smaller or greater numbers after this period.

It is not necessary to present detailed accounts of experiments in which the rough strains have been employed. The association between the presence of group antigen and excretion in the faeces was as close with the rough strains as with the smooth; but it was equally evident, taking the rough strains as a whole, that excretion in the faeces was less frequent than with the smooth strain. Reference to Tables I, II, III and IV in which the results of all experiments are summarised, will make these points clear.

[1] See footnote, p. 237.

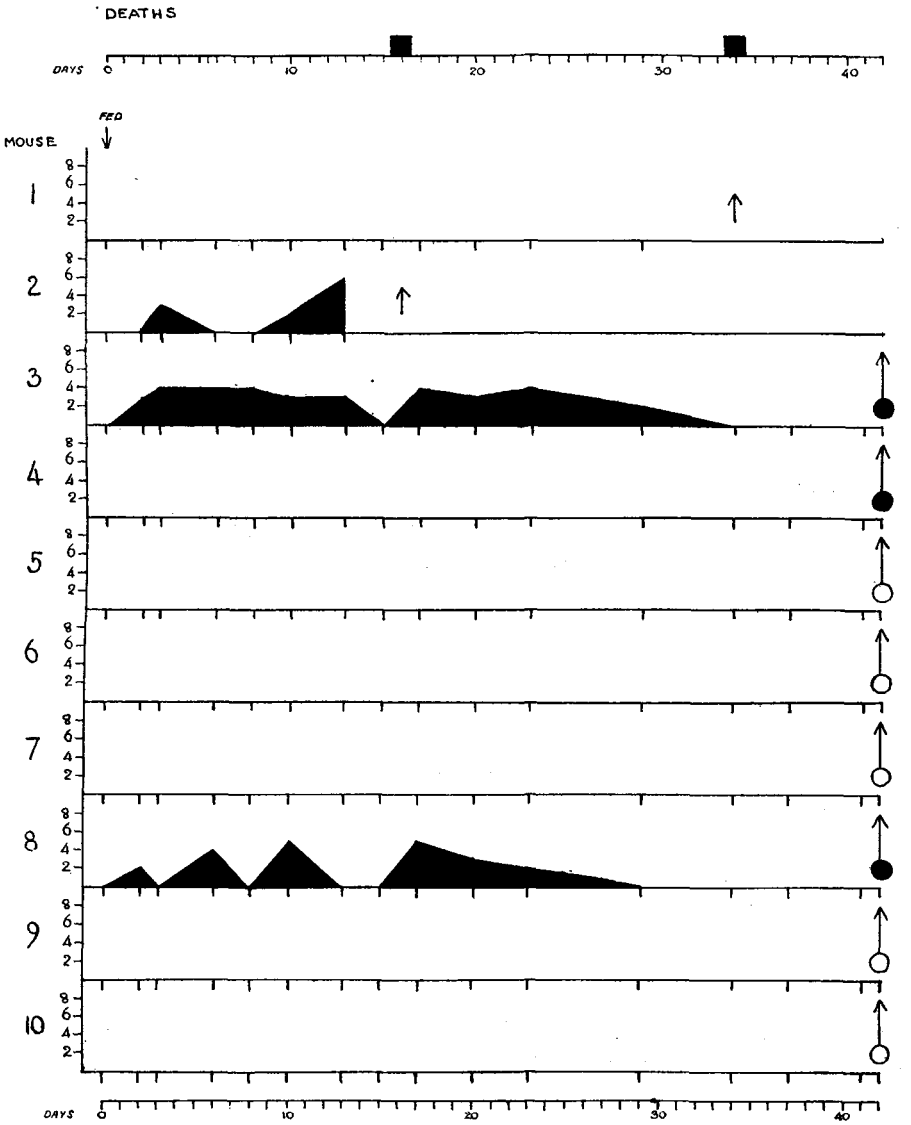

Chart V. Exp. *J.*

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EXPERIMENTS IN WHICH REPEATED ADMINISTRATIONS OF *B. AERTRYCKE* WERE CARRIED OUT.

Although the evidence obtained by single feedings of *B. aertrycke* seemed conclusive as regards the main point at issue, it seemed desirable to carry out a series of experiments in which an attempt should be made to establish faecal excretion of *B. aertrycke* by repeated administration by the mouth, using in some experiments cultures which we believed to be pure type strains, in others cultures which we believed to be pure group strains, and in some cases a mixture of the two. We expected that we should fail in our attempt to carry out repeated feedings with pure type strains, for the reasons we have already referred to. In several cases our expectation was realised, but we persisted, in these experiments in feeding type strains, in order to see whether type bacilli would appear in the faeces.

The following experiments will serve as examples:

Exp. Q. Chart VI. Five mice were fed on six occasions with cultures of *B. aertrycke* (smooth group strains). Each mouse, at each feeding, was given 0·02 c.c. of an 18 hours' broth culture of the strain employed. The mice were observed for 42 days, dating from the first feeding, with the following results:

Number of mice fed	5
Number of mice which excreted *B. aertrycke*	5
Number of specimens of faeces examined	75
Number of specimens of faeces positive	33
Excretion coefficient	155
Number of colonies from faeces tested by agglutination	139
Number of colonies reacting as Type	4
Number of colonies reacting as Group	36
Number of colonies reacting as Mixed	99
Number of mice which died	0
Number of survivors killed on 42nd day	4
Number of survivors with positive spleen cultures	1
Number of colonies from this culture tested by agglutination	10
Number of colonies reacting as Type	2
Number of colonies reacting as Group	0
Number of colonies reacting as Mixed	8

One mouse was kept alive for another purpose.

This experiment calls for no special comment.

Exp. W. Chart VII. Five mice were fed on five occasions with cultures of *B. aertrycke* (smooth type strains). The doses given were the same as in Exp. *Q*. The mice were observed for 42 days dating from the first feeding with the following results:

Number of mice fed	5
Number of mice which excreted *B. aertrycke*	1
Number of specimens of faeces examined	50
Number of specimens of faeces positive	1
Excretion coefficient	8
Number of colonies from faeces tested by agglutination	2
Number of colonies reacting as Type	2
Number of colonies reacting as Group	0
Number of colonies reacting as Mixed	0

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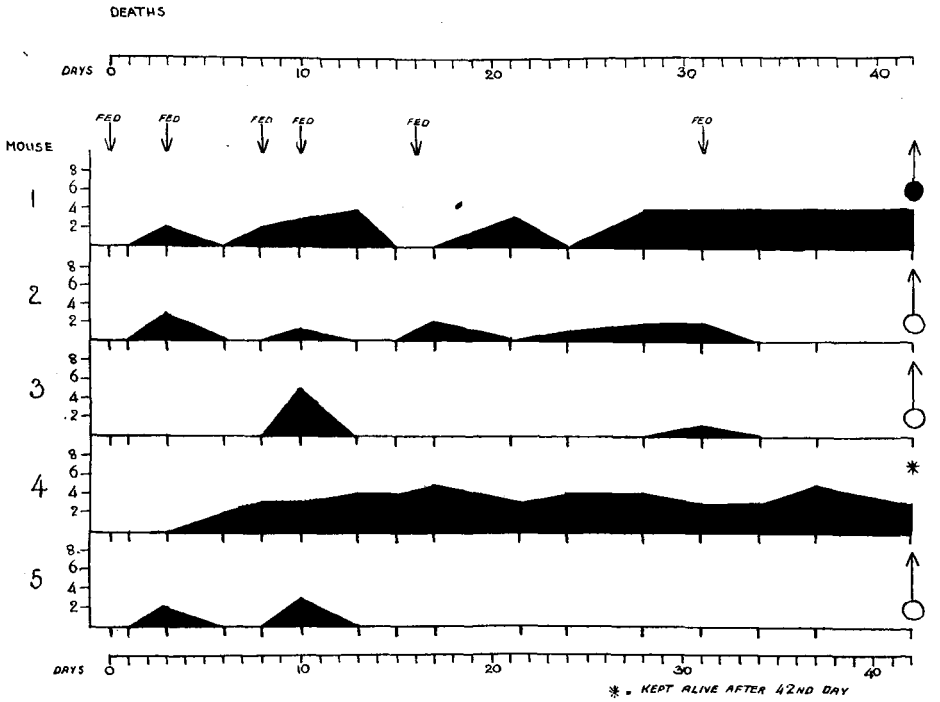

Chart VI. Exp. *Q*.

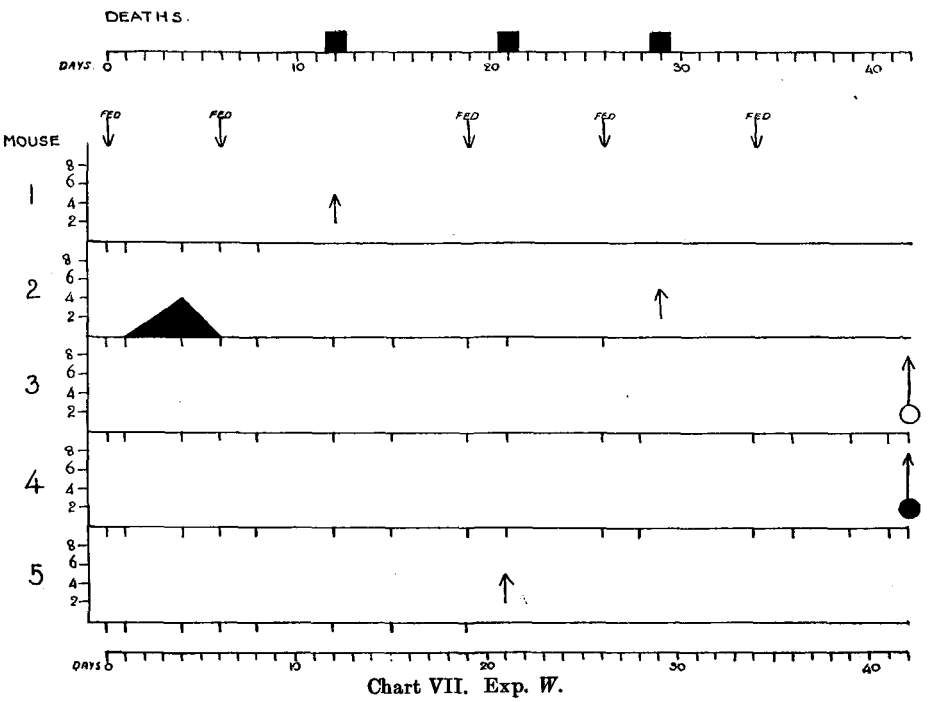

Chart VII. Exp. *W*.

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Number of mice which died		3
Number of mice positive on post-mortem examination		3
Number of colonies from these mice tested by agglutination ...		44
Number of colonies reacting as	Type	38
	Group	0
	Mixed	6
Number of survivors killed on 42nd day		2
Number of survivors with positive spleen cultures		1
Number of colonies from this culture tested by agglutination ...		10
Number of colonies reacting as	Type	2
	Group	0
	Mixed	8

This experiment may be contrasted with Exp. *Q*. It affords also another illustration of the fact that mice may be infected with type strains of *B. aertrycke*; and die of the infection, without ever excreting *B. aertrycke* in their faeces.

We may take this opportunity of emphasising a point, which must already have become obvious. It is quite clear, that, as a general rule, type bacilli do not change into group or mixed bacilli in the intestine. It is equally clear that, if type bacilli change into group or mixed bacilli in the tissues, the group or mixed bacilli so produced do not find their way into the intestinal tract.

Exp. P. Chart VIII. In this experiment an attempt to carry out six feedings with pure type strains resulted in failure. All details of the technique are the same as Exps. *Q* and *W*, the results were as follows:

Number of mice fed		5
Number of mice which excreted *B. aertrycke*		5
Number of specimens of faeces examined		64
Number of specimens of faeces positive		13
Excretion coefficient		59
Number of colonies from faeces tested by agglutination ...		47
Number of colonies reacting as	Type	4
	Group	1
	Mixed	42
Number of mice which died		2
Number of mice positive on post-mortem examination		2
Number of colonies from these mice tested by agglutination ...		14
Number of colonies reacting as	Type	2
	Group	0
	Mixed	12
Number of survivors killed on 42nd day		3
Number of survivors with positive spleen cultures		2
Number of colonies from these cultures tested by agglutination		20
Number of colonies reacting as	Type	12
	Group	0
	Mixed	8

Exps. *P* and *W* taken together afford an excellent illustration of failure to establish pure type bacilli in the faeces in spite of repeated feeding. In Exp. *P* the distribution of the serological varieties in the tissues differs from that in the faeces.

We may now consider the whole series of experiments, first from the point of view of the association between group antigen and faecal excretion, and then from the point of view of dosage.

Table I gives the more important figures for all experiments, omitting the

results of agglutination tests on the strains isolated from the faeces or from the tissues.

Table II gives the relevant details as regards the relation between group antigen and excretion, so far as the smooth strains are concerned. The first four experiments (*A–D*) are excluded because, as mentioned above, we were not at that time using an absorbed aertrycke serum, so that we could not differentiate the pure group strains from the mixed strains. The experiments in which repeated feedings were given are included with those in which the mice were fed on one occasion only, since no question of dosage is here involved. For the purpose of this analysis, the experiments in which smooth

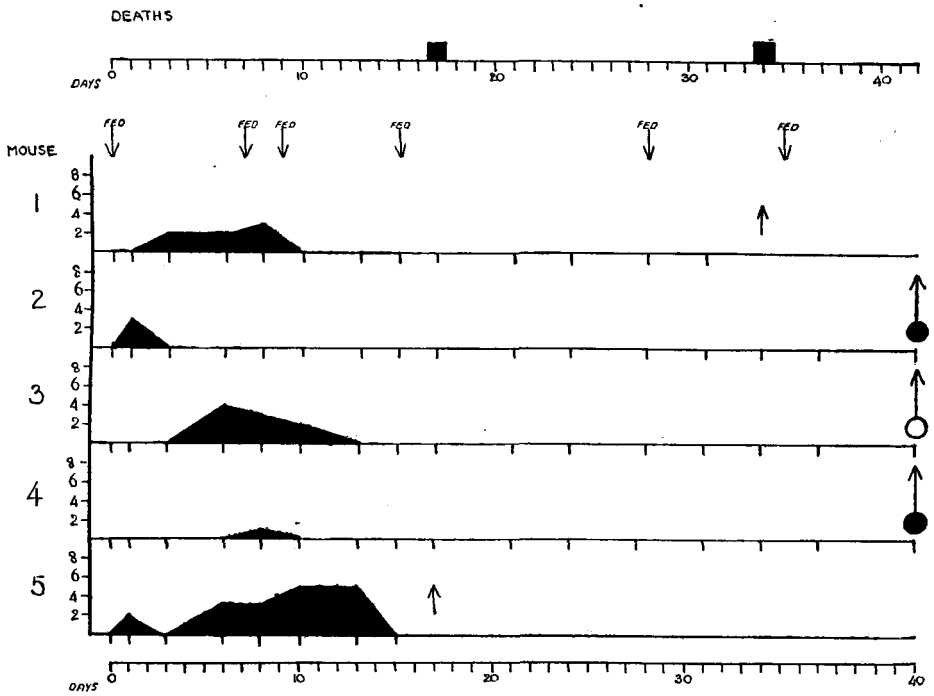

Chart VIII. Exp. *P.*

strains were employed are divided into three groups. Group 1 includes Exps. *E*, *F*, *L* and *W*, in which attempts were made to feed pure type cultures, and in which neither group nor mixed strains appeared in the faeces. The second group includes Exps. *G*, *H*, *M* and *Q*, in which attempts were made to feed pure group strains, and in which group or mixed strains appeared in the faeces. The third group includes Exps. *J*, *S*, *T* and *P*. In the first three of these experiments mixtures of type and group strains were fed to the mice. In Exp. *P* a supposedly type strain was administered, but mixed strains appeared in the faeces.

It will be seen from the table that the association of group antigen with faecal excretion is amply confirmed, whether we consider the results of feeding

Table I

Experiment	No. of mice	Strains fed	No. of times fed	Days observed	No. of mice excreting	Percentage of mice excreting	No. of specimens examined	No. of specimens positive	Percentage of specimens positive	Excretion coefficient	Total deaths	Specific deaths	Total deaths per cent.	Specific deaths per cent.	No. of survivors examined	Spleen cultures +	Spleen cultures −
E	10	S.T.	1	42	0	0	139	0	0	0	2	1	20	10	8	2	6
F	10	S.T.	1	42	3	30	144	5	3·47	13	2	2	20	20	8	0	8
L	10	S.T.	1	42	0	0	125	0	0	0	4	1	40	10	6	2	4
B	10	? S.T.	1	42	3	30	110	3	2·73	11	6	6	60	60	4	2	2
C	10	? S.T.	1	42	5	50	171	8	4·68	16	2	2	20	20	8	2	6
D	10	? S.G.	1	42	8	80	138	31	22·46	74	4	3	40	30	6	3	3
G	10	S.G.	1	42	6	60	134	15	11·19	38	3	3	30	30	7	2	5
H	10	S.G.	1	42	7	70	98	23	23·47	76	8	4	80	40	2	1	1
M	10	S.G.	1	42	2	20	131	2	1·53	2	2	2	20	20	8	1	7
A	10	S.M.	1	42	6	60	143	56	39·16	129	3	2	30	20	3*	0	3
J	10	S.M.	1	42	3	30	137	19	13·87	49	2	2	20	20	8	3	5
S	10	S.M.	1	42	5	50	113	23	20·35	78	4	4	40	40	6	4	2
K	10	R.M.	1	42	1	10	132	1	0·76	2	3	3	30	30	7	1	6
N	10	R.M.	1	42	4	40	151	12	7·95	19	1	1	10	10	9	1	8
O	10	R.M.	1	42	0	0	101	0	0	0	4	0	40	0	6	1	5
R	10	R.M.	1	42	2	20	124	14	11·29	33	2	1	20	10	8	3	5
W	5	S.T.	5	42	1	20	50	1	2	8	3	3	60	60	2	1	1
Q	5	S.G.	6	42	5	100	75	30	40	155	0	0	0	0	4†	1	3
T	5	S.M.	6	42	5	100	75	14	18·67	45	0	0	0	0	5	3	2
P	5	S.M.	6	42	5	100	64	13	20·31	59	2	2	40	40	3	2	1
U	5	R.M.	7	42	2	40	75	8	10·67	32	0	0	0	0	4†	3	1
V	5	R.G.	6	42	5	100	80	10	12·5	30	0	0	0	0	5	1	4
X	5	R.M.	5	42	2	40	64	2	3·13	13	1	1	20	20	3‡	2	1
Y	5	R.G.	6	42	0	0	85	0	0	0	0	0	0	0	5	3	2

S.T. = Smooth Type. S.G. = Smooth Group. S.M. = Smooth Mixed. R.G. = Rough Group. R.M. = Rough Mixed.

* = Two mice kept alive after 42nd day. † = One mouse kept alive after 42nd day. ‡ = One mouse accidentally killed.

Table II

Strain administered	Number of mice	Number of mice excreting	Percentage of mice excreting	Number of specimens examined	Number of specimens positive	Percentage of specimens positive	Number of colonies agglutinating as T.	Number of colonies agglutinating as G.	Number of colonies agglutinating as M.	Number of mice with positive tissue-cultures	Percentage of mice with positive tissue-cultures	Number of colonies from tissues agglutinating as T.	Number of colonies from tissues agglutinating as G.	Number of colonies from tissues agglutinating as M.
Smooth Type *E, F, L, W*	35	4	11·4	458	6	1·3	18	0	0	12	34·3	107	2	50
							100 %	0	0			67·29 %	1·26 %	31·45 %
Smooth Group *G, H, M, Q*	35	20	57·1	438	70	16	4	150	133	14	40	2	102	68
							1·39 %	52·26 %	46·34 %			1·16 %	59·3 %	39·53 %
Smooth Type + Group *J, S, P, T*	30	18	60	389	69	17·7	13	92	155	20	66·7	60	32	76
							5 %	35·38 %	59·62 %			35·71 %	19·05 %	45·24 %

T. = type. G. = group. M. = mixed.

Table III

Strain administered	Number of mice	Number of mice excreting	Percentage of mice excreting	Number of specimens examined	Number of specimens positive	Percentage of specimens positive	Number of colonies agglutinating as T.	Number of colonies agglutinating as G.	Number of colonies agglutinating as M.	Number of mice with positive tissue cultures	Percentage of mice with positive tissue-cultures	Number of colonies from tissues agglutinating as T.	Number of colonies from tissues agglutinating as G.	Number of colonies from tissues agglutinating as M.
All rough strains *K, N, O, R, U, V, X, Y*	60	16	28	812	47	5·8	19	10	145	21	35	69	18	80
							10·92 %	5·75 %	83·33 %			41·32 %	10·78 %	47·90 %

T. = type. G. = group. M. = mixed.

known type or group strains, or whether we compare the number of type, group and mixed strains isolated from the faeces in each of the three series of experiments.

It will be noted also that the ratio, between the percentage of mice excreting *B. aertrycke* in the faeces and the percentage yielding positive tissue cultures, is not constant for the three groups. Whereas 11·4 per cent. of the mice fed on type strains excreted *B. aertrycke* in their faeces, against 57·1 per cent. of those fed on group strains and 60 per cent. of those fed on mixed strains, it will be seen that 34·3 per cent. of those fed on type strains, 40 per cent. of those fed on group strains, and 66·7 per cent. of those fed on mixed strains harboured *B. aertrycke* in their tissues, including those that were killed on the 42nd day and gave positive spleen cultures.

The distribution of the type, group and mixed strains in the faeces differs from that in the tissues, where mice have been fed on mixed cultures. In Exps. *J*, *S*, *P* and *T*, of the strains isolated from the faeces only 5 per cent. were of the type variety. Of those isolated from the tissues 35·7 per cent. were pure type strains.

There seems to be ample evidence that, under the conditions here existing, the type variety can exist and multiply in the tissues far better than in the intestinal canal. Adopting the terminology suggested by one of us (Topley, 1923) in a previous report we might say that group strains or mixed strains of *B. aertrycke* are intragliscent and supragliscent, while type strains are intragliscent but not supragliscent.

Table III shows the results with rough strains, and may be dealt with very briefly. All figures for excretion are lower than with the smooth strains. We were not successful in feeding a pure type strain of the rough variety, although we had no difficulty in isolating strains which appeared to be purely of type character when tested by agglutination. The figures for the relative number of the three kinds of strain isolated from the faeces confirm those obtained with smooth strains, as regards the rarity of pure type strains, but mixed strains were far more numerous than group strains.

We may conclude this part of our report by giving the total figures for the distribution of the three serological varieties of *B. aertrycke* in the faeces. These are as follows:

Number of specimens of faeces examined		2660
Specimens positive		292
Number of strains tested by agglutination		1228
Number of these which reacted as	Type	89
	Group	248
	Mixed	891

Thus, 1139 of the 1228 strains, or 92·76 per cent. of all strains isolated from the faeces, possessed group antigen.

Table IV

Nature of bacterial strains	Dose in c.c.	Number of mice receiving this dose	Number of specimens of faeces examined	Number of specimens positive	Percentage of specimens positive	Excretion coefficient	Total deaths	Enteric deaths	Total mortality per cent.	Specific mortality per cent.	Number killed on 42nd day	Number of these with positive spleen cultures	Percentage of positive spleen cultures
All strains.	0·1	32	308	78	25·3	86	19	16	59·4	50	11*	8	72·7
Exps. *A, B, C, D, E, F, G, H, J, K, L, M, N, O, R, S*	0·01	32	430	58	13	36	9	8	28·1	25	22†	7	31·8
	0·001	32	458	50	10·9	40·6	9	5	28·1	15·6	22†	6	27·3
	0·0001	32	458	12	2·6	9	6	2	18·8	6·3	26	6	23·1
	0·00001	32	437	14	3·2	11·7	9	6	28·1	18·8	23	1	4·4
Smooth Type.	0·1	10	94	8	8·5	34	7	6	70	60			
Exps. *E, F, L, B, C*	0·01	10	140	3	2·1	5	3	3	30	30			
	0·001	10	156	2	1·3	5·1	2	1	20	10			
	0·0001	10	149	2	1·3	4·7	2	1	20	10			
	0·00001	10	150	1	0·7	3·3	2	1	20	10			
Smooth Group or mixed.	0·1	14	142	53	37·3	133·1	8	8	57·1	57·1			
	0·01	14	191	50	26·2	72·2	4	4	28·6	28·6			
Exps. *A, D, G, H, J, M, S*	0·001	14	197	44	22·3	82·2	5	3	35·7	21·4			
	0·0001	14	196	9	4·6	16·3	3	1	21·4	7·1			
	0·00001	14	168	13	7·7	27·4	6	4	42·9	28·6			
Rough Group or mixed.	0·1	8	72	17	23·6	61·1	4	2	50	25			
	0·01	8	99	5	5·1	10·1	2	1	25	12·5			
Exps. *K, N, O, R*	0·001	8	105	4	3·8	15·2	2	1	25	12·5			
	0·0001	8	113	1	0·9	1·8	1	0	12·5	0			
	0·00001	8	119	0	0	0	1	1	12·5	12·5			

*=2 mice kept alive after 42nd day.

†=1 mouse kept alive after 42nd day.

THE QUESTION OF DOSAGE.

In Table IV are set out the relevant facts as regards the effect of variation in dosage on the events which we have studied. In this analysis we have omitted those experiments in which the mice were fed on more than one occasion, but we have included the four experiments in which an unabsorbed aertrycke serum was used for the agglutination tests, since the inability to distinguish group from mixed strains does not affect the results from the present point of view.

We have given the results for all strains taken together and have then tabulated separately the figures for those experiments in which we employed (*a*) smooth type strains, (*b*) smooth group or mixed strains and (*c*) rough strains. The observations as regards positive spleen cultures in surviving mice were not sufficiently numerous to allow of their separation into the smaller groups.

In Chart IX we have plotted curves, showing the fall in the excretion coefficient with falling dosage (*a*) for smooth group or mixed strains, and (*b*) for smooth type strains. The latter curve has little real significance except that it demonstrates again that such strains are rarely excreted. It will be

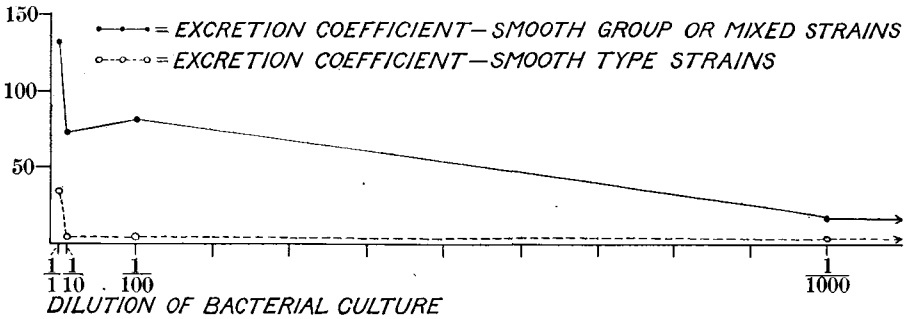

Chart IX

noted that, after the sharp fall when the dose is decreased ten times, the curve of excretion runs parallel to the base-line at a uniformly low level, indicating that it is only with relatively very large doses that any appreciable excretion occurs. The curve for the group and mixed strains is of some interest. It shows the same sharp fall when the dose is decreased ten times, but it remains at a relatively high level. It rises between the points corresponding to a ten-fold and hundred-fold diminution of the dose, and then falls very gradually towards the base-line, so that the excretion coefficient has been reduced to about 1/8th of its original value, when the dose has been decreased 1000 times. When the dose has further decreased to 1/10,000th of its original value, a point beyond the limits of the chart, the recorded value of the excretion coefficient actually rises again to about 1/5th of the figure for the undiluted culture. The total number of observations is of course too small to give any significance to small fluctuations, but we are probably safe in regarding our results as showing that the amount of bacterial culture, which we chanced

to adopt as our maximum dose, lay near to a point in the series of possible doses at which the influence of relatively small variations was rapidly changing in value; so that, while small decreases in the dose administered produced a marked reduction in the frequency with which the ingested bacilli became established in the intestinal tract of the host, further decreases, within wide limits, produced relatively little effect.

In Chart X we have plotted curves, for all strains, showing the relation between (*a*) decrease in dose and decrease in specific mortality, and (*b*) decrease in dose and decrease in the persistence of *B. aertrycke* in the tissues as judged by the percentage of positive spleen cultures 42 days after feeding. We have made an adjustment in the recorded figures in plotting the former curve. The specific mortality actually fell to 6·25 per cent. when the dose was decreased 1000 times, but it rose to 18·75 per cent. with a ten thousandfold reduction. No material significance attaches to this anomaly; the range of

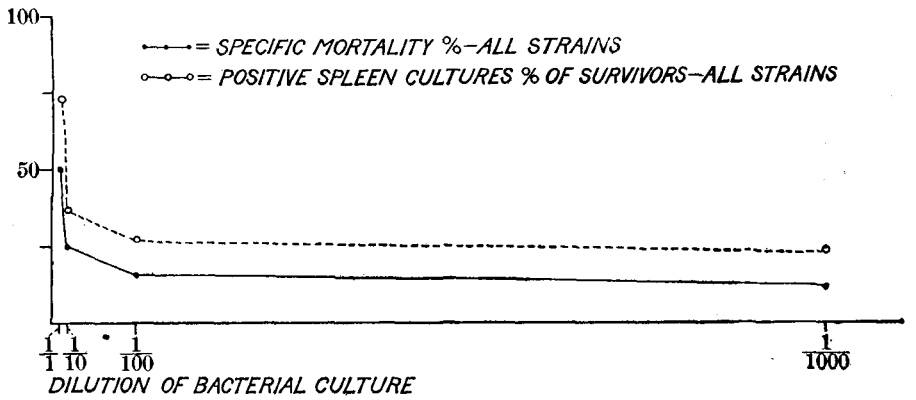

Chart X

probable variation in such small samples is large. It is impossible to record the value for the 1/10,000 dilution on the chart, and we have recorded the mean of the figures for the 1/1000 and 1/10,000 dilutions at the point corresponding to the former dose.

The points already referred to in connection with Chart IX are still more evident in Chart X, and we are inclined to think that the conclusions to be drawn from them are of some importance, and may help to explain certain differences between the results recorded by Webster (1923 *a*, *b*, *c*, *d* and *e*) and those which we have ourselves observed. Webster's usual practice has been to introduce 0·5 c.c. of a 1/100 dilution of an 18 hours' broth culture directly into the stomach through a silver catheter. His actual dosage thus corresponds to 0·005 c.c., a dose lying just below our second dilution, which corresponds to 0·01 c.c. There is, of course, the additional factor that Webster's dose passes directly into the stomach in a relatively large bulk of fluid, while the bacilli introduced by the method we have employed must first contend with the conditions in the buccal cavity. It seems to us very probable that

Webster has employed a dose, which, under the conditions imposed by his technique, approaches the limit at which the maximal effect of a single dose is obtained. This view is greatly strengthened by an experiment he records (Webster, 1923 *a*) in which varying dilutions of a broth culture were employed. Webster himself, in describing this experiment, speaks of the standard dose as "the massive lethal dose."

This is the dose which Webster has employed throughout, when testing different strains with regard to their capacity for giving rise to a fatal infection in mice; and he considers that a relatively constant percentage mortality among different groups of mice so treated is evidence that the strains of

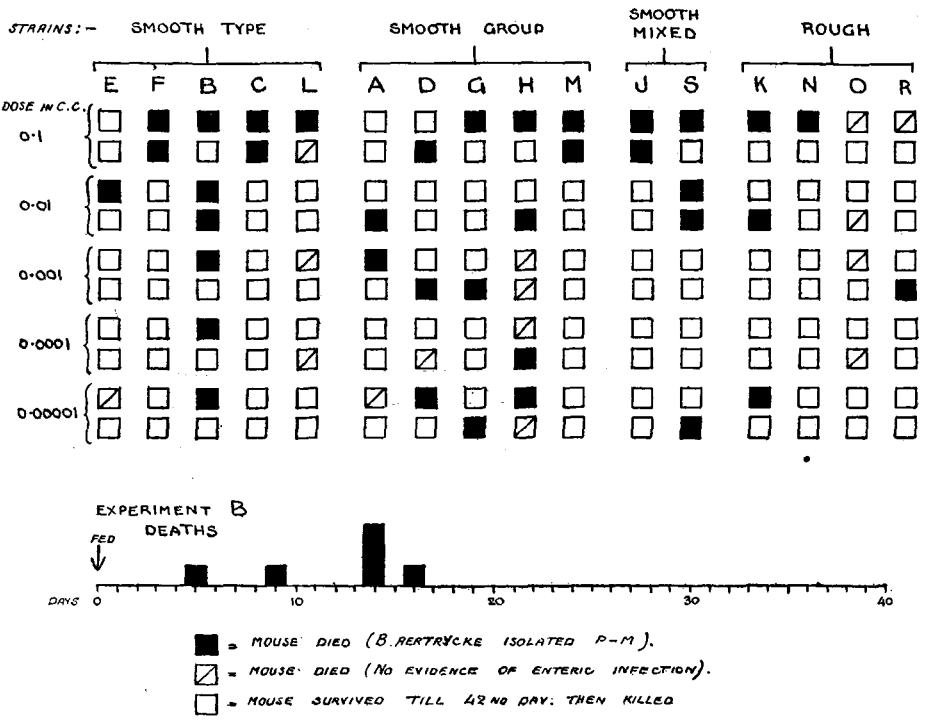

Fig. 1

B. aertrycke employed were constant in pathogenicity. It appears to us that this argument is open to objection. The observation of wide differences in the percentage mortality produced, when different strains of *B. aertrycke* are fed to mice under relatively constant conditions, may afford presumptive evidence that the strains vary in their power of producing a fatal infection. It by no means follows that the observation of a constant percentage mortality affords evidence of the absence of such variation. This would only be true if all strains examined produced like effects when they were tested over a wide range of doses. In the absence of the employment of a graded series of doses in testing each strain, the demonstration of differences in pathogenicity, if such

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exist, will depend on the selection of a dose which falls within the limits between which such differences are effective.

In Fig. 1 we show the distribution of deaths in relation to dosage in each of the 16 experiments available for such an analysis. These experiments were not planned to show variations in the death-producing powers of the strain employed, and they do not form suitable material for such a study, but we have little doubt that the strain used in Exp. *B* was actually more potent in this respect than the strains used in Exps. *F*, *L*, *J* and *M*. The mortality chart of Exp. *B*, reproduced at the foot of the figure, appears to us convincing, if we consider the time-distribution of the deaths and recollect that each mouse was kept in a separate cage so that no cross-infection could occur. Yet, did we only know the results of the maximum dose, we should not assign any pre-eminent position to this strain in the scale of pathogenicity.

Although this possible masking of differences in pathogenicity between different strains, by the employment of large doses of culture, may in part explain the results which Webster has obtained, we do not believe that it is the whole explanation. It seems probable to us that the effective dose, when the bacilli gain entrance to the buccal cavity is likely to differ widely from the effective dose as measured by injection into the stomach, and that quite other factors which may have a decisive effect in determining the issue under the former conditions, may be inoperative under the latter.

DISCUSSION.

It would be altogether premature, at this stage, to attempt any interpretation of the phenomena observed in studying epidemics among mice, by applying to them the conclusions arrived at in the present report. We may, however, enquire in how far we are justified in transferring these conclusions to the natural spread of enteric infection.

It seems probable that the almost complete absence of faecal excretion, following the oral administration of type strains of *B. aertrycke*, must possess some epidemiological significance; since it affords an example of selective localisation, which, we may reasonably suppose, will have some effect on the chance of spread of this particular variety of the parasite.

Our results as regards dosage would suggest that the size of any single dose ingested, using "size" in the sense of relative numbers of viable *B. aertrycke*, will not usually be a decisive factor in determining the course of events. We cannot, however, predict that the relations, which we have shown to exist between dosage and the events which we have studied, using pure cultures of *B. aertrycke*, will hold true when we deal with faecal material containing *B. aertrycke* as one constituent of an extensive microbial flora. Until data on this point are available, we must suspend judgment. The assumption that the infectivity of a given specimen of faeces could, in any case, be predicted from its relative content in viable *B. aertrycke*, as demonstrated by any method at our disposal, is quite unjustified in the absence of

experimental proof. This question will be discussed more fully in a subsequent report.

Although we should perhaps limit our conception of dosage, in the strict sense, to the number of viable *B. aertrycke* ingested on a single occasion; yet, as we have pointed out elsewhere, the idea of dosage may be expanded to cover many other factors. The distribution of *B. aertrycke* among the mice forming the population at risk, and the percentage of these mice which are excreting this organism in their faeces, must largely determine the probability of any single mouse ingesting *B. aertrycke* with its food; and we may reasonably expect that the distribution of *B. aertrycke* in the total bulk of excrement will afford a measure of dosage, not in terms of the numbers of bacilli ingested, but of exposure to risk of infection.

It may not be out of place to emphasise certain negative conclusions.

These experiments yield no evidence that the type, group or mixed varieties of smooth strains of *B. aertrycke* differ from one another in their power of producing infection and death. The difference between them is confined to their behaviour as regards excretion. There is some evidence that rough strains produce infection and death less readily than smooth strains; but we have, as yet, no evidence that rough strains play a part in epidemics of enteric infection.

CONCLUSIONS.

(1) Different strains of *B. aertrycke* show discontinuous variation in their behaviour as regards their excretion in the faeces of mice, following administration by the mouth. These variations are correlated with variations in antigenic character as judged by agglutination.

(2) Strains which contain group antigen, alone or in association with type antigen, tend to be excreted frequently and persistently.

(3) Strains which contain type antigen alone are rarely excreted in the faeces, and if excretion occurs it is transient.

(4) Mice may suffer from typical and fatal infection with type strains of *B. aertrycke* without ever excreting these organisms in their faeces in detectable numbers.

(5) Both type and group strains may persist in the tissues of mice which have ingested them for at least six weeks, without producing any apparent change in the animals' condition.

(6) As regards the production of a fatal infection, or persistence in the tissues as judged by the results of spleen cultures, there is no detectable difference between the two varieties.

(7) Rough strains of *B. aertrycke* are less frequently excreted in the faeces than are smooth strains. When, however, excretion occurs the great majority of the strains excreted contain group antigen. Mixed strains are far more numerous than pure group strains.

(8) The mortality produced by the administration of rough strains is less than that produced by the administration of smooth strains.

(9) Variation in dosage, over the range observed in these experiments, produces its greatest effect in the neighbourhood of the maximum dose employed. Relatively small decreases in these maximum doses cause a marked diminution in the effects resulting from them. Further reductions produce relatively quite small changes in the effects observed. This holds true whether we relate dosage to the subsequent excretion in the faeces, to the percentage mortality among the mice fed, or to the presence of the bacilli in the tissues of apparently healthy survivors.

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