THE INTERRELATIONSHIPS BETWEEN THE VARIOUS MEMBERS OF THE *B. ENTERITIDIS* —*B. PARATYPHOSUS* B GROUP OF BACTERIA.

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A REPORT TO THE MEDICAL RESEARCH COUNCIL.

(With 3 Charts.)

In a series of investigations on the epidemic spread of bacterial infection among mice (Topley, 1919, 1921), it has repeatedly been noted that, if an epidemic be started among a mouse-population by feeding certain animals on cultures of *B. gaertner* and subsequently introducing other susceptible mice into the cage, a varying proportion of these latter may succumb to infection with an organism morphologically and culturally identical with *B. gaertner*, but differing sharply from it in regard to its agglutination reactions. This organism has been referred to in previous communications as belonging to the *B. suipestifer* group, using that term to include *B. aertrycke* and other closely related types.

The object of the investigations already reported, and of others still in progress, has been to gain some knowledge of those biological laws which must govern the spread of epidemic disease. It has therefore been necessary to attempt an answer to the question whether we should regard an epidemic, during which mice have died from infection with each of these bacterial types, as a homogeneous outbreak, or whether we must consider each infection separately.

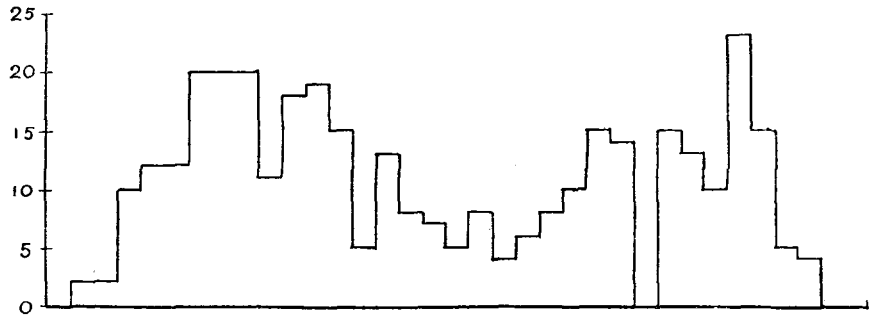
While we are far from suggesting that the serological differences which have been demonstrated are without significance, yet we believe that for broader purposes of analysis the whole series of deaths which occur under such circumstances should be taken together, a procedure which has already been adopted in considering the results of earlier experiments (Topley, 1921).

We have been led to this view by a consideration of the available evidence with regard to the interrelationships among the complex paratyphoid group, and of the ascertained facts regarding the serological complexity of such bacterial species as the Pneumococcus and the Meningococcus. We have also, in the course of the past three years, carried out a large number of experi-

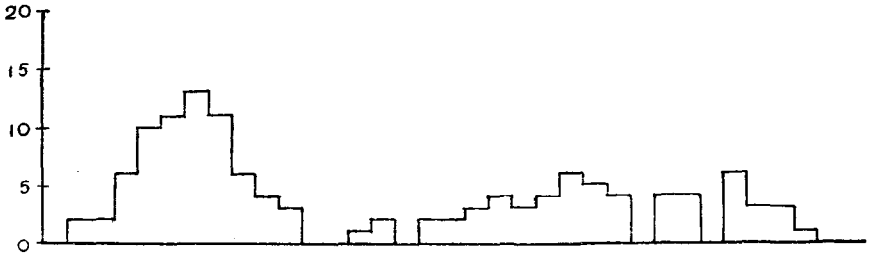
ments on points which have been brought prominently before us in our own work. It appears to us that the nomenclature and classification of the organisms of the paratyphoid-gaertner group may need some revision when viewed from a broader biological standpoint. For this reason the following facts may be worth recording.

THE EPIDEMIOLOGICAL EVIDENCE.

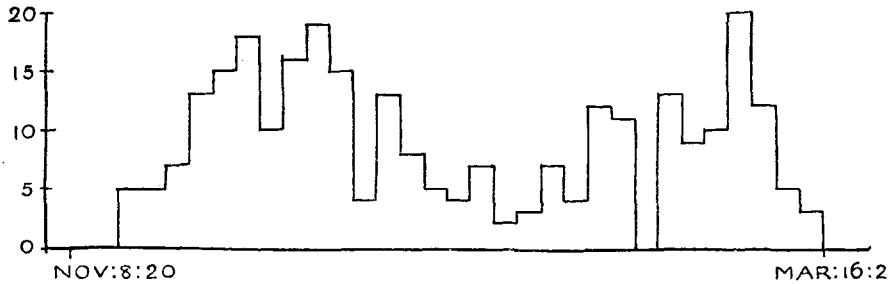
In general, if two epidemic diseases progress through a population during approximately the same time-interval, their course will be relatively independent, in the sense that a morbidity or a mortality curve will show individual waves each with its own maximum and minimum points. Certain individuals will contract both infections, and it is most unlikely that the progress of one infection is without influence on that of the other; but in the



Total deaths (excluding mice in which cause of death was undetermined).



Mice from which *B. suipestifer* was isolated post-mortem, alone or together with Pasteurella.



Mice from which Pasteurella was isolated post-mortem, alone or together with *B. suipestifer*.

CHART I.

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sense already referred to they may be regarded as separate events, and a mortality curve for the whole population concerned during the epidemic period may usually be analysed into two curves, having well-defined and separable phases.

That this is true of epidemics occurring under the experimental conditions which have obtained in our own work is shown by a consideration of Chart I, which illustrates the sequence of events following the accidental initiation of an epidemic due to an organism of the pasteurella group, in a cage in which an infection due to a paratyphoid organism had been progressing for many months. Throughout the period considered three normal mice were daily added to the cage. The deaths are charted in four-day intervals, and only those mice are included in which a full post-mortem examination clearly established the cause of death. Those animals in which such an examination revealed a double infection are included in the mortality chart referring to each disease.

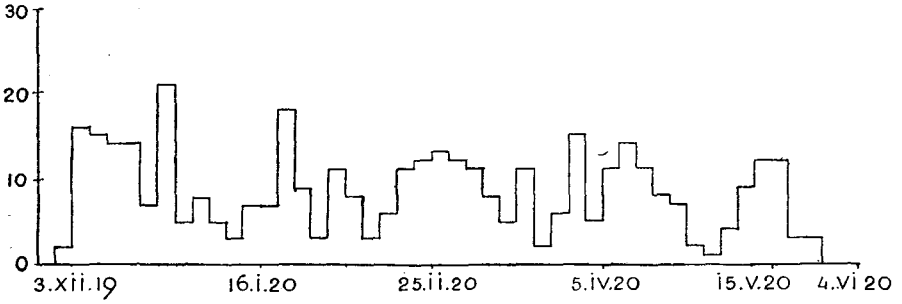
The results show that the two waves of mortality affecting the whole population are separable into two waves of suipestifer-infection and two of pasteurella-infection. The ascent of the first suipestifer wave commences before that of the pasteurella wave; its maximum is reached earlier, and it is falling while the latter is still rising. The ascent of the second suipestifer wave commences before the first pasteurella wave has fallen to its minimum, and again its maximum point antedates that of the latter.

In Chart II similar facts are given with regard to an epidemic, which was commenced by feeding mice on cultures of *B. gaertner* and subsequently adding susceptible individuals to the cage, at first irregularly and later at a constant rate. The bacilli isolated from the tissues of these mice were identical with regard to their morphological, cultural and fermentation reactions, but agglutination tests divided them into two groups, typical *B. gaertner* strains on the one hand, and, on the other, strains which were provisionally designated as *B. suipestifer*. Three colonies from the heart and three from the spleen were examined in every case. Many mice yielded pure cultures of one or other organism, but others yielded strains of both types.

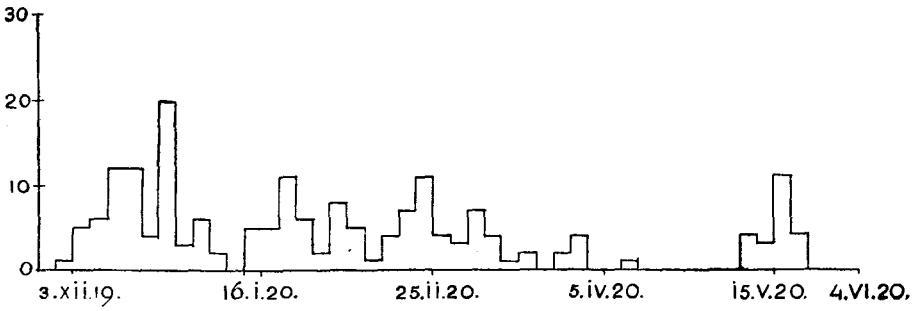
Examination of the chart shows that, in this case, the waves of mortality affecting the whole cage-population cannot be analysed into a regular series due to one type of organism, and a second series due to the other. In most of the successive waves each organism plays a part, nor is there any indication of separable maximal and minimal points. There is a general tendency for the relative frequency of isolation of the *B. suipestifer* strains to diminish as the experiment proceeds, and in the penultimate wave shown on the chart this type of organism apparently plays a very minor part. It is, however, well represented in the next and final wave.

It should perhaps be mentioned that smaller epidemics due to infection with these bacteria, and especially small outbreaks occurring spontaneously among laboratory stock, are more usually referable to organisms of one of

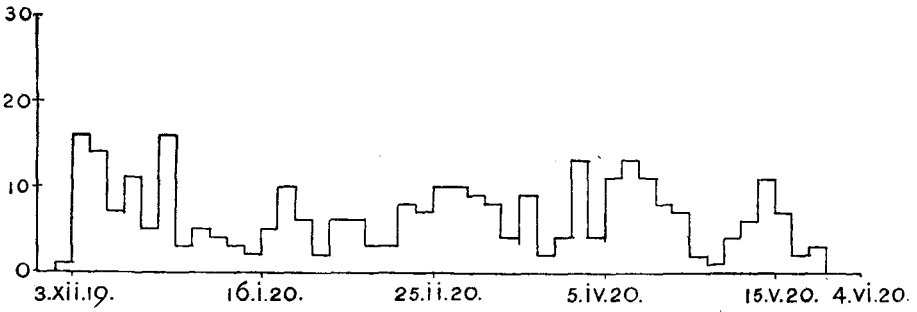
these serological types alone. If, however, the epidemic be of considerable proportions, and especially if large numbers of fresh susceptibles be exposed to infection, it frequently happens that both types are represented. It may be noted that, when the great majority of the mice yield strains of one type,



Mice from which *B. gaertner*, *B. suipestifer*, or both organisms were isolated post-mortem.



Mice from which *B. suipestifer* was isolated post-mortem, alone or together with *B. gaertner*.



Mice from which *B. gaertner* was isolated post-mortem, alone or together with *B. suipestifer*.

Chart II.

while strains of the other type are seldom recovered, these latter are commonly found among those animals which die in the early or closing stages of the epidemic. Chart III illustrates such distribution in two epidemics, each of which was started by feeding three mice on cultures of *B. gaertner*, and subsequently exposing other normal mice to infection.

We have not yet studied large epidemics, which have been started by infecting mice with strains of the *B. suipestifer* type; so that we do not know whether, under such circumstances, strains of the *B. gaertner* type would be frequently recovered from the tissues of those animals which succumbed. We have, however, investigated several spontaneous epidemics among mice, due to the former type of organism, but have never isolated any strain which has given the serological reactions of *B. gaertner*, although multiple agglutination with the three sera employed has been frequently observed.

It is of interest to compare these results with those observed in human epidemics of disease, and in certain outbreaks among laboratory animals, in which the causal organism is divisible into several serological types. Two bacterial parasites are especially well suited for enquiries of this kind, the Meningococcus and the Pneumococcus. The outbreaks of cerebro-spinal meningitis, which occurred among the troops in depôts during the recent war, afforded a large amount of material for such studies. The greater part of the evidence available, from the investigations carried out in this country,

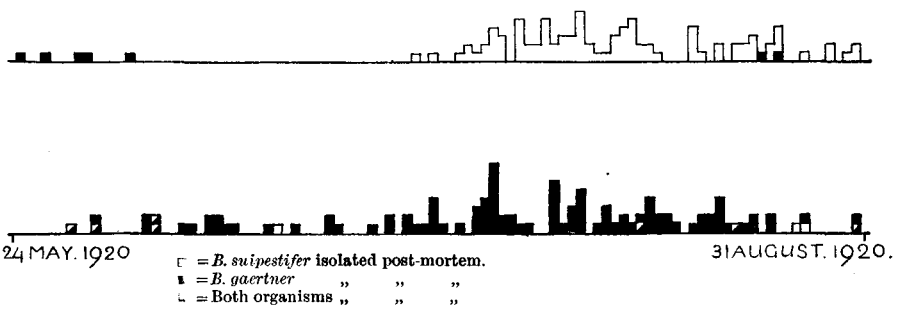


Chart III.

is contained or summarised in the series of reports issued by the Medical Research Council (1916–1920). The results recorded can be referred to only in the briefest manner, but the following facts may be regarded as definitely established. With regard to healthy carriers, it has been the universal experience of all observers that the rise in the meningococcal carrier-rate, which precedes an outbreak of meningitis, and the high level of this rate which is maintained during the epidemic period, concern not one of the serological types alone, but many or all of them; though one type usually stands out in marked predominance over the others. This multiplicity of serological types may be found not only in the same depôt, but among men sleeping in the same hut (Glover, 1918 *b*).

We are thus justified in stating that the pre-epidemic stage of that process, which results in the occurrence of an outbreak of cerebro-spinal meningitis, is marked by an increase of the general meningococcal population in the naso-pharynges of the human population concerned, and that, while one serological type of the parasite usually predominates, all types may, and

usually do, share in the invasion. When we turn to the strains isolated from the cerebro-spinal fluid of actual cases of disease we are on more difficult ground, mainly because the actual number of cases, which may be certainly grouped together as constituting a single outbreak, are in most instances relatively few. The results would seem to establish the following facts. In outbreaks involving a relatively small number of cases it is commonly found that all the Meningococci isolated belong to a single serological type. In some instances, however, cases which occurred in the same depôt, and were closely related in point of time, were referable to different serological types[1]. In such cases the predominant type of organism isolated from cases has tended also to be the predominant type found in carriers among the populations affected. This correspondence between prevalent carrier-types and prevalent case-types is well illustrated by the results obtained at Chatham by Armstrong and Tulloch[2], at Caterham (Glover, 1918*a*, 1920), and in the figures for the London Command in general quoted by Glover (1920).

In this connection we may note a result obtained in an experimental epidemic already reported (Topley, 1921). Fifteen mice, which had passed through a considerable epidemic in which the great majority of deaths had been due to infection with *B. suipestifer*, but in which *B. gaertner* had played a minor rôle, were killed and examined post-mortem. The animals were apparently in perfect health, and no death had occurred in the cage during the previous fourteen days. From the tissues of nine of these mice *B. suipestifer* was isolated, in eight cases in pure culture and in one together with *B. gaertner*. Thus the type of organism which was predominant during the epidemic was also more frequently isolated from the tissues of those animals which survived it.

An epidemic of cerebro-spinal fever on a far larger scale is reported by Olitsky (1919). This outbreak, which occurred among a Chinese civil population, was responsible for 1041 diagnosed cases, and Olitsky estimates that the total number affected was probably in the neighbourhood of 2500. Pure cultures of Meningococci were obtained from the cerebro-spinal fluid in 60 cases. Of these, 59 were obtained during the height of the epidemic. Agglutination tests identified 56 of these 59 strains as Parameningococci, which are stated to correspond to Gordon's Type I. The other three strains were identified as belonging to an "irregular Paratype," which is stated to correspond to Gordon's Type III. One strain was obtained from a case which occurred a few weeks after the main epidemic had come to an end. Serologically it was found to be a Meningococcus of the "normal type," stated to correspond to Gordon's Type II.

With regard to the Pneumococcus, we are faced with the same difficulties as in the case of the Meningococcus. Many of the outbreaks in which the serological type of the cocci isolated has been recorded, have involved rela-

[1] *Med. Res. Council Special Reps.* 1918 and 1920, and especially Fildes and Baker, 1918.
[2] *Ibid.* 1920.

tively few cases; but some records, notably those of Lister (1913, 1916, 1917) on pneumonia on the Rand, have dealt with far larger numbers of cases. It is, however, difficult to obtain information from the available records with regard to the serological type of any considerable series of strains, isolated under such circumstances that we may safely regard them as being the causative agents in a homogeneous outbreak of disease. The outbreaks of pneumococcal infection associated with the recent pandemic of influenza have afforded further evidence as to the presence of several serological types among the Pneumococci infecting the population concerned, but here the primary epidemic process is due to some other organism.

Some of the following facts, however, are not without significance. It is clear from the account given by Lister (1917) of the incidence of the various types of Pneumococci among the native mine-workers, that several types were isolated from cases occurring within a relatively short period in the same mine, which apparently formed well-defined groups. In a careful investigation among the native workers on the Crown Mines, who had received prophylactic inoculation against the three serological races, which were responsible for a majority of the cases of lobar pneumonia on the Rand, Lister (1917) investigated the type of Pneumococcus isolated in 80 out of 82 successive cases of lobar pneumonia. He found that the three types against which protective inoculation had been carried out were entirely unrepresented in this series of cases. Fifty of the 80 strains isolated were separable into seven distinct groups, while 30 remained unclassified.

Opie, Freeman, Blake, Small and Rivers (1919) report an outbreak of pneumonia at Camp Funston among negro drafts. Among 34 cases, in which the Pneumococci were investigated by agglutination, four separate groups were involved, and 13 of the 34 strains fell into the entirely heterogeneous Group IV. The many reports which have been published by American observers on the Pneumococci isolated during influenzal epidemics, all agree in recording several different serological types among the organisms isolated, whether from blood cultures, from sputum, or from the lung tissue at post-mortem examination. Again, while the very thorough studies of Stillman (1917) indicate unmistakably the correspondence between case-type and prevalent carrier-type of Pneumococcus (so far as the patient and his immediate contacts are concerned), yet they do not support the view that the case-type is always uniform, even in quite small outbreaks.

Finally, we would select for reference an interesting record of a spontaneous outbreak of pneumonia among monkeys being kept as laboratory stock. This outbreak is reported by Blake and Cecil (1920), and involved the death of 36 monkeys among 98 animals which had arrived in the laboratory in two batches. Pneumococci were isolated in 28 cases, and all were found to belong to the heterogeneous Group IV. Agglutinating sera were prepared against three strains, and with these sera 21 of the 28 strains were retested; nine fell into one group, five into another and seven remained unclassified.

The conclusions which appear to be justified from a consideration of the facts recorded in this section may be summarised as follows. The available evidence regarding epidemics of disease, caused by a bacterial species which shows subgroups separable from one another by serological reactions, indicates that representatives of all or many of these subgroups take part in the essential process on which the epidemic depends. In small outbreaks it is a common experience for all clinical cases of the disease in question to be referable to infection by organisms belonging to the same serological subgroup, but in larger outbreaks this uniformity is frequently absent. If parallel examinations be carried out on healthy contacts and non-contacts, living in the epidemic area, it will almost always be found that the serological subgroup responsible for all, or for the majority of the clinical cases, is the predominant group among the total number of strains of the parasitic species concerned, which are isolated from the population at risk.

The phenomena observed in experimental epidemics, in which *B. gaertner* and *B. suipestifer* have played a causative rôle, would suggest that these organisms are related to one another in the same way as are the serological subgroups of such bacterial species as the Pneumococcus or the Meningococcus.

EVIDENCE FROM THE IDENTITY OF THE LESIONS PRODUCED.

In the course of the past three years we have carried out post-mortem examinations on over 2000 mice which have died as the result of infection with *B. gaertner* and *B. suipestifer*. We do not propose to give any detailed account of our study of the lesions observed beyond stating that the findings of previous workers have been largely confirmed, and in some directions amplified. We may emphasise however that certain of the tissue changes, and especially perhaps the multiple necrotic areas so frequently found in the liver, are striking and typical. Examination of this considerable number of mice has entirely failed to reveal any constant difference, which would separate those animals which have died from infections with organisms of one serological type from those which have succumbed to the attack of the other.

EVIDENCE WITH REGARD TO THE SEROLOGICAL RELATIONSHIP OF *B. GAERTNER* AND BACILLI OF THE SUIPESTIFER GROUP.

There has been a general consensus of opinion among most observers that, while *B. paratyphosus* B, *B. aertrycke* and *B. suipestifer* show close serological relationship, so that absorption tests are frequently necessary to separate them on the grounds of their agglutination reactions, *B. gaertner* is sharply marked off from the members of this group by its direct response to agglutination with specific sera. Evidence has not, however, been wanting that this separation may not be so absolute as has been supposed. Sobernheim and Seligmann (1910) studied certain strains of *B. paratyphosus* B, which became inagglutinable with their specific antiserum, and at the same time

acquired the property of being agglutinated with a gaertner antiserum. The serum produced by inoculation of these strains agglutinated *B. paratyphosus* B but not *B. gaertner*.

Since the time when we first noted that organisms with the serological reactions of *B. suipestifer* were being isolated from mice dying during an epidemic started by feeding with *B. gaertner*, it has been our routine procedure to test each strain isolated against high-titre agglutinating sera prepared against *B. gaertner*, *B. aertrycke* (Mutton) and one of the earliest strains of the *B. suipestifer* type isolated in the course of this work. Blood from the heart and spleen-pulp have been smeared directly on to plates of agar or MacConkey's medium, or primary broth cultures from these sources have been plated out after preliminary incubation. From the plate-cultures so obtained from each mouse, three colonies derived from the heart and three from the spleen have been subcultured into broth. After 24 hours' incubation at 37° C. these subcultures have been killed by diluting with normal saline containing 0·5 per cent. formalin and heating to 55° C. for one hour, a second subculture having first been made in order that the strain might be available for further study if desired. Heat has been employed in addition to formalin for killing the cultures because, when several hundred strains were being examined weekly, it was impossible to test each suspension for sterility by subculture. Previous experience had shown us that the procedure adopted ensured uniform sterility without rendering the suspensions in any way unsuitable for our purpose.

From the first, it was obvious that the serological complexity of the strains isolated was considerable. Almost every imaginable variety of result has been obtained in direct agglutination tests, and there is little doubt that, had additional sera been employed, still greater complexity would have been revealed. A large number of strains, on first isolation, agglutinate to titre with the gaertner or suipestifer serum and are quite unaffected by the other two. Many strains agglutinate with two of the three sera, and when this is the case the combination is almost always gaertner and aertrycke, or suipestifer and aertrycke. Very few suspensions have agglutinated with gaertner and suipestifer sera, while remaining unaffected by the aertrycke serum; and further subcultures from such strains have always resulted in the isolation of both the gaertner and suipestifer types, so that they were presumably mixed strains from the outset. Not infrequently a strain has been agglutinated by all three sera. When multiple agglutination occurs, the strain concerned may be agglutinated to titre by all three sera, much more commonly by two of them; or only one serum may produce this result, the others giving agglutination in lower dilution. All strains which have behaved in this way have been further subcultured and retested. The results have been uniform and striking. In every case, a strain which on first isolation agglutinated with more than one of the three sera employed has, after a very varying number of subcultures, yielded a strain which has agglutinated to titre with one serum

and remained entirely unaffected by the others. It may be noted here that we have never isolated a strain which has permanently displayed the serological characters of *B. aertrycke* (Mutton). Still further subcultures may again yield strains showing multiple agglutination, but if the process be persisted in the cultures will again revert to the specifically agglutinable type. Moreover, strains of *B. gaertner* or *B. suipestifer*, which show typical agglutination to titre with one of the corresponding antisera, never give rise to strains showing typical agglutination to titre with the other. In practice, it is usually possible to distinguish the serological type to which a strain actually belongs at the first agglutination test. Either it will agglutinate to titre with the gaertner or suipestifer serum, but not with the other two sera, or it will form large flocculi with one serum, and much smaller and tighter clumps with the other. In such cases further subculture always yields a strain agglutinating only with that serum, which originally caused agglutination to titre or the formation of large flocculi. This variation in the type of the clumps produced has been noted by many workers, and has recently been extensively studied by Arkwright (1921) in his investigations on saline-agglutinable strains.

The figures quoted in Table I may be considered typical of the results we have obtained. The upper horizontal line gives the agglutination titres, obtained with one of three colonies picked from a plate inoculated with a 24 hours' broth culture from a mouse, which died during an epidemic of mouse-paratyphoid. Below are given the results obtained on retesting subcultures

Table I.

		Sera		
Date	Strain	Gaertner	Aertrycke (Mutton)	Suipestifer
9. xii. 19	Sp. A	6400	6400	6400
6. ii. 20	Sp. A1	6400	800	800
	Sp. A2	6400	1600	1600
	Sp. A3	6400	800	–
	Sp. A4	6400	–	400
	Sp. A5	6400	1600	400
13. ii. 20	Sp. A1 A	6400	3200	1600
	Sp. A1 B	6400	–	–
	Sp. A1 C	6400	1600	800
	Sp. A1 D	6400	–	–
	Sp. A1 E	6400	–	–
17. ii. 20	Sp. A1 A1	6400	–	–
	Sp. A1 A2	6400	–	–
	Sp. A1 A3	6400	–	–
	Sp. A1 A4	6400	–	–
	Sp. A1 A5	6400	–	–

Note:—The five strains tested on 6. ii. 20 were obtained from five separate colonies from a plate inoculated with strain Sp. A tested on 9. xii. 19. Similarly the five strains tested on 13. ii. 20 were derived from strain Sp. A1 tested on 6. ii. 20, and those tested on 17. ii. 20 from Sp. A1 A6 tested on 13. ii. 20. The figures indicate the highest dilution of serum giving well-marked agglutination.

– = No agglutination at a dilution of 1/400.

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of this strain at later dates. The strain was subcultured once only between successive tests, and plate cultures were prepared from which colonies were picked after 24 hours.

Further investigation soon made it clear that the occurrence of strains showing multiple agglutination was not dependent on passage through the tissues of the mice. A culture of *B. gaertner* obtained from the Lister Institute in 1918, which on previous examination had agglutinated with its specific antiserum but not with the other sera employed, was frequently subcultured in broth over a period of many months.

Plate cultures were prepared at intervals from which ten separate colonies were picked, subcultured into broth, and tested against the three antisera indicated above. It is unnecessary to tabulate the results, for they were in all essential respects similar to those already recorded. Multiple agglutination was frequently met with, sometimes to such an extent that there was no indication of the real nature of a given bacterial culture from the results of a single test. In this series of experiments it was by no means unusual to find strains, derived from this culture of *B. gaertner*, which entirely failed to respond to the specific antiserum, but which agglutinated to a high titre with the aertrycke serum employed. At other times, strains were obtained which failed to agglutinate with any of the three sera, but further subculture invariably revealed the true nature of the strain concerned.

A single instance of this kind may be quoted. From a subculture of the original strain, a culture was prepared from a single bacterial cell by a method described elsewhere (Topley, Barnard and Wilson, 1921). From this culture a plate was inoculated from which ten colonies were picked. The resulting broth cultures were tested against the three antisera, but gave no trace of agglutination with any of them. Two of these were further subcultured and treated in the same manner. In one case all the ten subcultures agglutinated with the gaertner and the aertrycke serum, but while they agglutinated to titre with the latter they were only acted upon by their specific antiserum in much lower dilutions. In the second case three out of six subcultures were agglutinated to a high titre by the aertrycke serum, but not at all by the specific antiserum. One of these latter strains was again plated out, and six colonies were subcultured and retested. They all agglutinated to titre with the gaertner serum, but were only acted upon by the aertrycke serum in lower dilutions.

The real nature of such a gaertner strain, which has become inagglutinable by its own antiserum while responding to the action of an aertrycke serum, is at once revealed by absorption tests. Absorption of an aertrycke serum by such a strain leaves the titre against *B. aertrycke* entirely unchanged, while diminishing or abolishing its action on the abnormal gaertner strain. If, however, the same strain be employed to absorb a gaertner serum, the agglutinins both for *B. gaertner* and for the aberrant strain may be entirely removed.

While it would be wearisome to repeat details of similar variations among strains which originally showed multiple agglutination, but finally reacted as pure suipestifer strains, or starting as pure suipestifer strains came to show multiple agglutination, it may be stated that all the observations noted above have been paralleled among such strains, with the exception that we have not carried out absorption experiments in these cases.

In view of the recent observations of Arkwright (1921) on the separation of saline agglutinable and inagglutinable strains of *B. dysenteriae* (Shiga), it was natural to enquire whether the variant strains we have studied showed similar phenomena. Our technique was not well-suited for revealing such relations, had they been present, but it was possible to make certain observations along these lines. Thus the roughness or smoothness of the colonies on plates, and the type of growth in the broth subcultures, were noted in large series of cases. The form of the colonies varied widely, and in general a given plate culture tended to show all rough or all smooth colonies, but either character might be continued through many generations, or suddenly give place to the other. So many intermediate types of colonies were met with that a classification into rough and smooth was frequently very arbitrary. The growth in broth was in most cases of the type usual with organisms of this group, with uniform turbidity and slight deposit. Occasionally, however, an entirely different kind of growth occurred, a heavy, almost granular deposit collecting at the bottom of the tube, while the supernatant fluid remained clear or only slightly cloudy, but often showed a film of surface growth. On shaking such a culture a uniform suspension was obtained, which proved quite suitable for agglutination tests. This type of growth was particularly common after prolonged subculture, with frequent transplants from broth to broth. It was especially with such strains that the more aberrant type of agglutination was likely to occur, and it seemed at first as though some relation might be established between the two phenomena. More extended experience has shown that, while such cultures are more likely to depart widely from the normal type in regard to their agglutination reactions, especially in the direction of loss of specific agglutinability associated with the acquirement of agglutinability by a heterologous serum, yet they often react in a perfectly normal manner; while equally wide variations may occur in cultures which show a perfectly normal type of growth. We have entirely failed to establish any relation between the roughness or smoothness of the colonies and the subsequent behaviour of subcultures in agglutination tests.

To sum up these results, it may be said that examination of large numbers of strains has shown that the serological distinction between *B. gaertner*, *B. aertrycke* and *B. suipestifer* is less sharp than has been commonly supposed, so far at least as direct agglutination tests are concerned. An organism of one type may acquire the property of being agglutinated to titre by one or both of the heterologous sera. It may become inagglutinable by its specific antiserum at the same time as it acquires the property of agglutinability by

a serum specific against one of the other types, though this is a far less common occurrence. While this interrelationship is clearly shown by direct agglutination, the same procedure, repeated on many successive subcultures, always reveals the true nature of the strain, and the indication so obtained is confirmed by absorption tests.

THE QUESTION OF MUTABILITY.

The observations described above raise once again the question whether the serological types dealt with are bacterial mutants, in the sense that one type may be repeatedly derived from another under natural or experimental conditions. It would be an immense satisfaction to obtain an unequivocal answer on this point, but no adequate evidence has yet been brought forward. The matter has been debated, as regards this group of organisms, by Schmitt (1911), Mühlens, Dahm and Fürst (1909), Gurney-Dixon (1919), Jordan (1920), and others.

We have ourselves observed large numbers of strains over considerable periods of time, up to two years; and have subjected many of them to highly abnormal conditions of environment, such as prolonged storage in distilled water or normal saline, growth at 45° C. over many months, and prolonged growth on agar at different temperatures, with subcultures of the papillae from the large colonies which form under these conditions. Without entering into details we may say that we have completely failed to obtain a strain which, after adequate examination, could be placed in any other group than that proper to the strain from which it was originally derived.

We have also carried out feeding experiments on considerable numbers of mice, testing the strains recovered from the internal organs after death, or obtained by killing the animals while in apparently good health at various intervals after feeding. In the majority of cases, mice fed on cultures of *B. gaertner* have yielded cultures of this type alone, and similarly with mice fed on cultures of *B. suipestifer*. Occasionally, however, we have isolated one of these serological types from the tissues of a mouse fed on cultures of the other. It is clear that such instances of the isolation from animal tissues of a strain, showing different serological reactions from those of the strain administered, cannot be regarded as satisfactory evidence of the mutational origin of the former strains.

THE RELATIONSHIP BETWEEN THE MEMBERS OF THE PARATYPHOID-ENTERITIDIS GROUP.

This thorny question has long been a subject of controversy between different schools of bacteriologists. Any adequate résumé of the literature would be far beyond the compass of this communication. The excellent survey of Uhlenhuth and Hübener (1913) gives a summary of the evidence acquired prior to that date, and considers it from the point of view of those who would divide the group into two subgroups only, one confined to

B. enteritidis (Gaertner), and the other including *B. paratyphosus* B, *B. aertrycke*, *B. typhi-murium*, *B. suipestifer*, and a host of allied organisms recovered from the tissues and excreta of sick or healthy animals.

Bainbridge (1912) and Bainbridge and O'Brien (1911) have insisted on the distinction which may be drawn between *B. paratyphosus* B, on the one hand, and *B. aertrycke* and *B. suipestifer* on the other, by means of absorption tests. The identity which they found between the two latter types is probably invalidated, as pointed out by Tenbroeck (1920 *a*), by the fact that their *B. suipestifer* strains were of German origin, and may well have been of the type which would be referred to by recent American observers as swine-typhus bacilli. The results of Bainbridge and O'Brien have, however, led to a very general use of *B. suipestifer* and *B. aertrycke* as synonymous terms in this country; and it is in this wide sense that the former has hitherto been used by us. Schütze (1920), by an extended use of absorption tests, has recently erected several additional subgroups within the general *B. paratyphosus* B group.

The most valuable recent additions to our knowledge of this complex group have been derived from the studies of various American workers, including among others, Jordan (1917, 1918 *a*, 1918 *b*, 1920), Jordan and Victorson (1917), Krumwiede and Kohn (1917), Krumwiede, Pratt and Kohn (1916 *a*, 1916 *b*, 1917), Krumwiede, Kohn and Valentine (1918), Krumwiede, Valentine and Kohn (1919), Smith and Tenbroeck (1915), Tenbroeck (1918 *a*, 1918 *b*, 1920 *a*, 1920 *b*), Murray (1919), Muslow (1919), Rettger and Koser (1917), Meyer and Boerner (1913), Good and Corbett (1913), Gage and Martin (1916), and Winslow, Kligler and Rothberg (1919). In the following brief summary only those papers are referred to, which deal with those organisms that have been less thoroughly studied, or with facts recently brought forward.

The careful studies of fermentation reactions and serological relationships, which have been undertaken, have left a picture of the whole group which may be presented somewhat as follows. The organisms which comprise it, with certain exceptions noted below, have the following characters in common. They are gram-negative, non-sporing, motile bacilli. They ferment dextrose, maltose, mannite, xylose and rhamnose with the formation of acid and gas. They do not ferment lactose, saccharose, salicin, raffinose, dextrin nor inulin. They produce transient acidity in litmus-milk, but later give rise to a markedly alkaline reaction. They reduce neutral red. They do not form indol nor do they liquefy gelatine.

This group, according to the researches so far conducted, may be split up into the following subgroups:

(*a*) *B. paratyphosus* B possesses the general characteristics of the group, rapidly ferments dulcite, ferments arabinose and inosite, produces blackening of lead acetate and reduces the fuchsin in the serum-water medium recommended by Krumwiede, Pratt and Kohn (1917). It is readily distinguishable

from *B. enteritidis* (Gaertner) by direct agglutination, and from other members of the group either by direct agglutination or by absorption.

(*b*) *B. suipestifer* possesses the general characteristics of the group, ferments dulcite slowly or not at all, does not ferment arabinose nor inosite, does not blacken lead acetate medium, and does not reduce the fuchsin in serum-water medium. This organism may be distinguished from *B. enteritidis* by direct agglutination, and from *B. paratyphosus* B and allied species, sometimes by direct agglutination but more commonly by absorption tests. According to Reed and Carroll (1900) and Tenbroeck (1920 *b*) *B. icteroides* (Sanarelli) belongs to this subgroup.

(*c*) *B. paratyphosus* C, so named by Hirschfeld (1919), who isolated it during an epidemic of paratyphoid fever in the Balkans, but more generally known as Hirschfeld's Bacillus, to distinguish it from Uhlenhuth's *B. paratyphosus* C, to which apparently it has no relation. It has all the general characteristics of the group, ferments arabinose and dulcite and blackens lead acetate. As regards its fermentation reactions therefore it cannot be differentiated from *B. paratyphosus* B and *B. enteritidis*. A recent study by Tenbroeck (1920 *b*) has shown, however, that serologically it is identical with *B. suipestifer*, as judged both by direct agglutination and by absorption tests.

(*d*) *Paratyphoid bacilli of animal origin.* This large subgroup includes a great number of strains derived from the tissues and excreta of animals, either healthy or diseased. There is good evidence that the bacteria concerned are in many cases the actual cause of the disease in question, while in other cases they should perhaps be regarded as secondary invaders. It is also established that several animal species are subject to severe epidemics, in which these organisms play a causal rôle. These strains possess the general characters of the group, they ferment dulcite and arabinose, while their reaction in inosite appears to be irregular. It is noteworthy that Tenbroeck (1920 *a*) obtained indefinite results with this substance, and was unable to establish any differentiation between *B. paratyphosus* B, *B. enteritidis* (Gaertner) and these animal paratyphoid bacilli on the basis of their fermentation reactions. They blacken lead acetate medium and reduce the fuchsin in serum-water medium. They are distinguishable from *B. gaertner* by direct agglutination, and from other members of the group by absorption tests. When specific sera are prepared against individual strains of this group, there appears to be a correlation between the degree of agglutination and host-origin. It is probable that *B. aertrycke* belongs to this subgroup, and also those *B. suipestifer* strains of German origin which were studied by Bainbridge and O'Brien (1911). The bacilli isolated from mice dying during our own experiments, and hitherto referred to as *B. suipestifer*, have all the characteristics of this subgroup and should be placed in it.

(*e*) *B. enteritidis* (Gaertner) possesses all the characteristics of the group. It ferments dulcite and arabinose, but not inosite; though the value of this latter substance for the purpose of differentiation has still to be firmly estab-

lished. It blackens lead acetate medium and reduces the fuchsin in the dextrose-serum-water medium of Krumwiede, Pratt and Kohn. It is separable from most other members of the group by direct agglutination, but shows definite serological relationship with *B. pullorum* and *B. gallinarum*, from both of which it may be separated by absorption tests (Smith and Tenbroeck, 1915, and Muslow, 1919).

(*f*) *B. abortus equi*, a paratyphoid organism which appears to be responsible for epidemic abortion in mares, has been studied by Smith (1893), Kilbourne (1893), Good and Corbett (1913), de Jong (1913), Meyer and Boerner (1913), van Heelsbergen (1914), Murray (1919), and Fitch and Billings (1920). It possesses the general characters of the group, ferments dulcite and arabinose but not inosite, fails to reduce lead acetate and does not reduce the fuchsin in serum-water medium. On agar it forms a distinctive, dry and brittle growth. Serologically, it is readily distinguishable from other members of the group.

(*g*) *B. pullorum* is regarded by many authorities, but not by all, as the cause of bacillary white diarrhoea in chicks. It was isolated by Rettger (1900), and has been studied by Smith and Tenbroeck (1915), Gage and Martin (1916), Rettger and Koser (1917), Krumwiede and Kohn (1917), Hadley, Caldwell, Elkins and Lambert (1917), Hadley, Elkins and Caldwell (1918), Muslow (1919) and others. According to the reports of most of those who have studied this organism, it differs from the groups mentioned above by its non-motility, its initial production of acidity in milk followed by a very slow change to alkalinity, and by its failure to ferment xylose and maltose. Muslow (1919), however, reports uniform production of acid in xylose, with very variable gas-production, and doubts the reliability of the reaction in litmus-milk, as a means of differentiating between this organism and *B. gallinarum*. It ferments arabinose, but not dulcite nor inosite. As regards its action on lead acetate there is a distinct conflict of statement. Muslow (1919) reports that all strains examined by him produced rapid blackening. Winslow, Kligler and Rothberg (1919), summarising and confirming the results of other workers, report negative reactions. These authors, on the grounds of the reaction in litmus-milk and the absence of fermentation of xylose, would place this organism in the same group as *B. paratyphosus* A, rather than in the *B. paratyphosus* B-*B. enteritidis* group; and there is clearly much to be said for this classification. Serologically, however, *B. pullorum* appears to be identical with *B. gallinarum*, as tested by direct agglutination or by absorption of agglutinins. Both these organisms show a close serological relationship to *B. enteritidis* and also to *B. typhosus*, absorption being required to differentiate them from these organisms. There is also a definite serological relationship between *B. pullorum* and *B. gallinarum* on the one hand, and *B. abortus equi* on the other, but direct agglutination generally affords a sufficient means of differentiation.

(*h*) *B. gallinarum*, described by Klein (1889) as the cause of an epidemic

of fowl-typhoid in England, is almost certainly identical with the bacillus described by Moore (1895) as *B. sanguinarium*, and has since been studied by several workers, including Pfeiler and Rehse (1913), Smith and Tenbroeck (1915), Pfeiler and Roepke (1917), Hadley, Caldwell, Elkins and Lambert (1917), Hadley, Elkins and Caldwell (1918), and Muslow (1919). It departs from the general characters of the group by being non-motile, by failing to produce gas in the various test-media and by attacking dextrin. On the other hand, it produces the typical alkaline reaction in litmus-milk and ferments xylose. For these reasons Winslow, Kligler and Rothberg (1919) are inclined to place it in the group with *B. paratyphosus* B and *B. enteritidis*, in spite of the absence of gas-formation. It ferments arabinose and dulcite but not inosite. With regard to the blackening of lead acetate medium, there is the same conflict of opinion as in the case of *B. pullorum*. Its serological reactions have already been discussed, and it is somewhat surprising that organisms, showing such wide differences with regard to their fermentation reactions as do *B. pullorum* and *B. gallinarum*, should show serological identity.

DISCUSSION.

It has been suggested above that the two types of organism isolated during the course of experimental epidemics in mice, one of which has given the serological reactions of *B. enteritidis* (Gaertner) and the other those of the animal-paratyphoid group, might be regarded on epidemiological grounds as being related to one another in the same way as are the various serological types of Meningococci or Pneumococci. It has been further shown that the demarcation between the two types, afforded by agglutination tests, is not so sharp as has been supposed; though we have obtained no evidence suggesting that either type permanently loses its serological characters, or exchanges them for those of the other.

A large number of strains belonging to each type have been tested with regard to their fermentation reactions. All test-substances recorded by recent observers have been employed, with the exception of inosite, and of the dextrose-serum-water with the Andrade indicator. We were unable to obtain a supply of inosite at the time when the fermentation reactions were being carried out, and the results recorded by Tenbroeck (1920 *a*) seemed to us to throw some doubt on its reliability as a means of differentiation. In all the media we employed the reactions of all strains have been identical, and have conformed to those generally ascribed to the subgroups in question.

Under these circumstances it would appear that the relationship between these two bacterial types would be most accurately reflected in the nomenclature by giving them a common name, and indicating the serological subgroup by a convenient suffix. The existence of this relationship has, indeed, been fully recognised in a recent report dealing with bacterial classification (Winslow, Kligler and Rothberg, 1919), in which *B. enteritidis*, *B. suipestifer* (strictly so-called), the *B. paratyphosus* B forms and *B. gallinarum* are in-

cluded in a separate subgroup of the colon-typhoid group of bacteria. It appears to us that the actual relationship is closer than is suggested by such a grouping. Employing the terminology which is, almost inevitably, adopted in bacteriological classification, we would suggest that a single "specific" name be given to the organisms comprising certain of these subgroups. From the strictly scientific point of view, the use of the term "species" and the application of specific names are, perhaps, hardly to be defended, when we consider our almost complete lack of knowledge of the actual life-history of the organisms with which we are dealing. Though the terms we use suggest analogies between the groupings we recognise and those set up in other branches of biology, yet the characters upon which our classification is based may be fundamentally different.

With these reservations, we may call to mind certain resemblances between the relationships existing among bacterial types, and those known to obtain elsewhere in nature. The larger groups of the Linnaean species are now generally acknowledged to be divisible into very numerous elementary species, as was first urged by Jordan (1853), and later insisted upon as a fundamental phenomenon by de Vries (1901). Yet the reality of the Linnaean species has not been seriously questioned, nor would a classification which raised elementary species to specific rank, as ordinarily understood, yield a possible nomenclature for systematic use. It is at least tempting to draw an analogy between serological types of bacteria and elementary species, as understood by de Vries. No one who has had extended experience with such bacterial groups as the Meningococci, the Pneumococci and the paratyphoid bacilli, will readily deny the reality of serological differentiation. The question is rather the significance which is to be attached to the differences so demonstrated. It appears to us that there is evidence in support of the view that the unit-group of such bacteria, which functions in natural parasitism, is larger than the serological subgroups, and probably includes many of them.

It seems possible that the most urgent requisite for a better understanding of bacterial parasitism is an increased knowledge of bacterial ecology. For this reason it would perhaps be better to note those minor yet constant differences, which undoubtedly occur in strains which are perpetuated under laboratory conditions, without necessarily utilising them in systematic classification. The present tendency appears definitely to be in the direction of testing bacterial strains as to their action upon an ever-widening range of possible food materials, and subjecting them to more and more subtle serological tests, giving to each strain which is separable by such means the rank of type, race or species as the case may be, and in most cases bestowing upon it a distinctive name.

We are far from minimising the value of such detailed studies. We have, indeed, obtained experimental evidence, which would seem in some measure to explain the significance of such serological subgroups in the spread of bacterial infection. This evidence we hope to publish in a future report. The

question immediately before us, however, is one of classification, and especially of nomenclature. The matter is to some extent urgent, for the present condition is chaotic; and the recent reports of the American Society of Bacteriologists (Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith, 1917) indicate clearly that some attempt at systematisation, however provisional and imperfect, will have to be made. It is from this point of view that we would plead for a broad outlook, so that our nomenclature may indicate those groupings which are most fundamental in the natural existence of the organisms which we study, and may not depend mainly on small differences observed under artificial cultivation. It would perhaps be an advantage if the Linnaean admonition "Varietates levissimas non curat botanicus" were borne in mind by the bacteriologist, so far at least as classification is concerned.

We are inclined to uphold the view that the large group of bacteria, which we have been considering, including at least *B. paratyphosus* B, *B. enteritidis* (Gaertner), Hirschfeld's bacillus, *B. aertrycke*, and the majority of the paratyphoid organisms isolated from animals, should receive a single specific name. If we include *B. enteritidis* in this group, and it is our main thesis that it should be so included, then this name holds priority over all others, with the exception of *B. suipestifer*. It appears to us that the latter title is far less suitable, for at least two reasons. It was applied to the organism in question by Salmon and Smith (1886) in the belief that it was the specific cause of swine-fever, so that its retention would perpetuate a view now generally held to be incorrect. Again, while we should be inclined to make the specific group as wide as possible, it must be recognised that the constancy of those fermentation reactions, which differentiate the true *B. suipestifer* from *B. paratyphosus* B and *B. enteritidis*, may possibly be held to necessitate the inclusion of the former in a different specific group. We would therefore suggest that the name of *B. enteritidis* be employed as a specific title for the bacterial types referred to above, with the possible exclusion of *B. suipestifer*, and the probable exclusion of *B. pullorum* and *B. gallinarum*.

It would be desirable to add to the specific name another, indicating the serological subgroup to which a strain belongs. This subgroup might be regarded as a variety. Thus we might employ the names Gaertner, Schottmüller and Hirschfeld to denote the three well-defined subgroups discovered by these observers, while the names suipestifer and aertrycke might be used to denote these two varieties, though the latter term would then have to include a large number of paratyphoid strains of animal origin. It would be a distinct advantage if we could make the names of our varieties denote their host-origin, and with further knowledge this may become possible, but at present some of the best marked serological differences seem to cut clearly across the lines of specific parasitism.

CONCLUSIONS.

For the reasons set out above, it seems probable that the relation between *B. enteritidis* (Gaertner) and many of the members of the *B. paratyphosus* B group of bacteria is similar to that existing between the serological subgroups of such bacterial species as the Meningococcus or the Pneumococcus. It is therefore suggested that, for purposes of classification and nomenclature, *B. enteritidis* should be included in this group, and that its name might well be applied to the whole group of which it forms a part, both on account of priority and of suitability. The subgroup, or variety, to which a given strain belongs, provided that this is sufficiently definite and constant, should be indicated by adding a name designating the subgroup or variety in question.

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