OBSERVATIONS ON THE BACTERIOLOGY OF PARATYPHOID FEVER AND ON THE REACTIONS OF TYPHOID AND PARATYPHOID SERA.

By A. E. BOYCOTT, M.A., M.D.,

Assistant Bacteriologist to the Lister Institute; Fellow of Brasenose College.

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During the last eight years the existence has been established of a sub-acute bacterial septicaemia clinically resembling typhoid fever, but associated with the presence in the body of organisms which are closely allied to Gaertner's food-poisoning bacillus and amply distinct from the *B. typhosus*. The cultivation of a "paracolon" bacillus from the blood of a patient "with all the clinical features of typhoid fever," by N. B. Gwyn in 1898, represents the first clear diagnosis of a case of this paratyphoid fever, though the same organism had been already isolated by Achard and Bensaude (1896) from the urine of a case of apparent typhoid and from a purulent arthritis following a similar illness, and Widal and Nobécourt (1897) had found it in a thyroid

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abscess arising in a patient apart from any symptoms of general infection. The observations of Cushing (1900), followed by those of Buxton (1902), Libman (1902) and others (see Johnston 1902 and Pratt 1903), established the position of paratyphoid fever as a definite, and not very uncommon, disease in America, while Schottmüller (1900, 1901), by the systematic application of the method of blood-culture in cases of "typhoid," showed that a similar condition obtained in Germany. Sporadic cases, with occasional epidemics (de Feyfer and Kayser 1902, Fischer 1903, Schottelius 1905) have since been described from many parts of the world, and the disease is probably of general distribution. In this country, however, only four cases seem to have been recorded, if we exclude such indefinite instances as those mentioned by Parsons[1] and Cautley[2]. Craig and White (1902) isolated from the spleen *post mortem* of "a case of continued fever resembling enteric, and due to the *B. enteritidis*," an organism which was probably a paratyphoid rather than a Gaertner; Hume's (1902) "bacillus L," obtained from the stools and urine of a "typhoid," seems to have belonged to the paratyphoid group; and Savage (1905) has recently described two cases of the disease, in one of which *B. typhosus* was isolated from the urine. As will presently be pointed out, paratyphoid bacilli cannot be definitely differentiated from other closely related forms without the use of agglutination tests with sera of known origin. It is unfortunate that these tests have not been applied in any of these four cases. It may therefore be worth while to put on record here three instances of paratyphoid fever which have been met with during the past year, together with two further cases in which the existence of a similar condition was highly probable though not fully proven.

I. *Methods of investigation employed.*

In dealing with organisms, such as the present, where distinctions of some narrowness have to be drawn and where details which at present appear immaterial may hereafter become of importance, it is desirable to give some account of the methods and standards employed.

(1) *Media.* For plates, the lactose-bile-salt neutral-red agar of MacConkey (1905, p. 334) has been exclusively used, and has been found altogether satisfactory. For identification, besides the customary media, much use has been made of peptone water containing neutral red or

[1] *British Medical Journal*, 1905, vol. I. p. 305.

[2] *British Journal of Children's Diseases*, 1905, vol. II. p. 241.

litmus with 0·5 or 1 per cent. of various carbohydrates, alcohols, and glucosides, and filled into Durham's fermentation tubes.

(2) *Isolation.* Direct plating of stools may be successful, but, as always in working with such material, *B. coli* is much in the way. A differential medium which will encourage the growth of paratyphoid more than that of *B. coli* is of assistance. Such a medium is found in the dulcite-bile-salt peptone water of MacConkey. It is characteristic of the whole group of paratyphoid and food poisoning organisms that they ferment dulcite freely[1]. This fermentation is accompanied by proliferation, so that in dulcite broth dulcite-fermenters tend to overgrow non-dulcite-fermenters, just as in lactose broth the *B. coli* still further outnumber the non-lactose-fermenters. In the following experiment, approximately equal quantities of paratyphoid B[2] (fermenting dulcite but not lactose) and "*coli* 1" (fermenting lactose and dulcite but not cane-sugar: *B. coli communis*) or "*coli* 4" (fermenting lactose but not dulcite or cane-sugar: *B. acidi lactici*) were inoculated into plain broth, lactose peptone water, and dulcite peptone water; after 20 hours' incubation at 37° C. plates were made and the relative numbers of each organism now present determined.

	P.c. found on plates	
	Coli	Paratyphoid
Paratyphoid + *coli* 1 incubated in plain broth	19	81
,, ,, ,, lactose	96	4
,, ,, ,, dulcite	18	82
Paratyphoid + *coli* 4 incubated in plain broth	95	5
,, ,, ,, lactose	99	<1
,, ,, ,, dulcite	1	99

The dulcite-fermenters present in faeces comprise about half the *B. coli* (A. C. Houston (1904, p. 544) and A. MacConkey (1905)), certain members of the food-poisoning group isolated by H. de R. Morgan (1905), and two or three other unnamed varieties of "*coli*-like" organisms: the last two are not abundant. They are therefore far less abundant than *e.g.* lactose- or dextrose-fermenters, and by means of this preliminary differential culture in dulcite media paratyphoid B was isolated from the stools in two cases in which direct plating had given altogether negative results.

[1] This important reaction seems to have been first noted for hog-cholera by Voges and Proskauer (*Zeitschrift für Hygiene*, 1898, vol. XLVIII. p. 20) and for paratyphoid by Conradi, Drigalski and Jürgens (1903).

[2] The strain used was "*Potts*" (see below). All three organisms were recently isolated from human faeces.

The highly pathogenic relations of these organisms suggests another convenient method of isolation, and after inoculation of guinea-pigs with infected faeces (and especially with cultures of faeces in dulcite broth) the organisms may often be obtained in abundance from the heart-blood. There is, however, evidence that similar organisms may be recovered from the blood of animals inoculated with material undoubtedly free from any such bacillus; thus A. MacConkey (1905, p. 343, *note*) obtained bacilli culturally resembling paratyphoid B[1] after the inoculation of dead typhoid bacilli. It is clear from this that the method cannot be used unless corroboration be obtained without the use of animals.

Circumstances have in the present cases precluded the use of direct cultivation of the patient's blood which seems to be at once the simplest and most accurate method of isolation.

(3) *Agglutination reactions* have in all cases been observed microscopically in the hanging drop. This was in the present instance necessary owing to the small quantities of serum often available; apart from this, however, it seemed desirable to make use of the more delicate method. Broth cultures of the organisms grown for about 20 hours were used. Such preparations as dead formalinised cultures might have afforded a more uniform test-standard, but a series of preliminary experiments, in which the agglutinabilities of such preparations and of fresh living cultures were compared, gave results distinctly unfavourable to the dead bacilli as regards delicacy as well as rapidity of reaction. It appeared too that the difference between the dead and living bacilli was not relatively the same with different sera (human), some sera showing no difference, while others had much less action on the dead than on the live organisms. The same serum shows much less (and absolutely quite little) variation when reacting with a number of live cultures grown under similar circumstances of time, temperature, and medium. The most desirable temperature for growth varies with the different organisms used, and must be determined by experiment; thus one typhoid ("Guy's") at 20° grows into long threads, while another ("Lincoln") is practically non-agglutinable if grown at 37°. In the same way some paratyphoid and food-poisoning organisms grown at 37° are very prone to spontaneous agglutination; this is not present in a growth of 5 or 6 hours at 42° or

[1] Probably belonging to the hog-cholera group which produces one form of pseudo-tuberculosis of guinea-pigs.

of 24 hours at 20°. In other cases it seems to be immaterial whether the culture has been at 20°, 37°, or 42°. The customary series of serum-dilutions in 0·9 per cent. NaCl at which observations were made was 1 in 20, 1 in 50, 1 in 100, 1 in 500, 1 in 1,000, 1 in 5,000, and 1 in 10,000: the wide spacing of the higher dilutions is justified by the unfortunate lack of sharpness in the end-point which is inherent in the reaction[1]. Observations were made after the mixtures had stood for half to one hour at room temperature; in all comparative tests the time-limit was the same throughout the series. Control preparations were made in all cases. The degree of reaction has been noted in five grades:—complete clumping = +++; nearly complete but some free bacilli = ++; a fair number of small clumps with many free bacilli = +; a few clumps composed of perhaps not more than three or four organisms = tr.; same as control = 0. The agglutination limit of sera failing to react at 1 in 20 is generally described as 0. The refusal to recognise reactions other than those of a "complete" kind fails to take into account phenomena which control experiments show to be of a perfectly definite nature. Since the range of serum-dilution required to reduce a "complete" to a "trace" reaction varies a great deal with different combinations of bacilli and sera, this may lead to altogether erroneous results. The degree of reaction must be estimated by comparison with the appearances presented by the most complete reaction obtainable with the given organism and kind of serum. This differs a good deal in different combinations. Thus Gaertner and paratyphoid B show only loose, relatively open aggregations of bacilli with any dilution of their respective immune sera. These might be regarded as only partial reactions if judged by the conventional standard of the dense clumps formed by typhoid bacilli in typhoid serum. The highest dilution of a serum at which a reaction is obtained has throughout been taken as quantitatively representing the content of the serum in agglutinin.

(4) *Absorption tests.* The absorbing organisms have been agar cultures of (generally) two days' growth; the serum dilution 1 in 10 to 1 in 500, and the time two or three hours at 37°. Nothing is gained by longer contact except a rapid deterioration of the serum; as a matter of fact one hour seems to be ample. In some instances the agglutination limits of the serum were redetermined after absorption; with a combination,

[1] As illustrating the quantitative limitations of the phenomenon the observations of G. Dreyer and A. J. Jex-Blake (*British Medical Journal*, 1904, vol. II. p. 564) should be consulted.

however, of many absorbing organisms and many agglutinable bacilli, the test becomes very extensive, and in the majority of cases the diluted serum has been mixed with each organism (added if necessary in successive amounts) until it ceases to react with that bacillus or reacts with that bacillus alone: its reaction with the other bacilli is then observed qualitatively at a definite dilution. By thus saturating the serum by the addition of an excess of bacilli, difficulties are avoided in the measurement of comparable quantities of organisms differing in size, texture and fluidity, which are inherent in the method by which "equal" quantities of bacilli are added to equal quantities of serum and the relative disappearance of agglutinin estimated. For many of the problems this simpler method will not of course suffice; for the applied purposes of diagnosis it is very satisfactory.

If the agglutination limits are determined after absorption, some allowance is necessary for the inevitable and unknown dilution produced by the addition of water with the absorbing bacilli and for the deterioration of agglutinating power which is caused by incubation at 37° for several hours (cf. Park and Collins 1904, p. 500). The influence of this latter factor seems inconstant: it may be very marked.

II. *Description of organisms concerned.*

The following is a list of the strains of the various standard organisms which have been used[1]: the names by which they are referred to below are in italics.

A. Typhoid.

1. *Guy's:* the standard agglutinating strain of this laboratory.
2. *Lincoln:* recently (June 1905) isolated from the urine of a typhoid convalescent.
3. *A. E. Wright:* old laboratory strain.
4. *Delépine:* „ „ „

The cultural and agglutination (including agglutinogen) reactions of all four strains were typical. The fermentative reactions were: acid without apparent gas in dextrose, maltose, laevulose, galactose, sorbite, and mannite, but no change in lactose, cane-sugar, dulcite, raffinose, arabinose, erythrite, salicin, amygdalin or inulin. They differed somewhat in their action on litmus milk; all produced a definite acidity

[1] The cultural characters and agglutination reactions of most of these have recently been fully described by H. de R. Morgan (1905) and fuller details of the fermentative properties of some of them are given by A. MacConkey (1905).

in 24 hours; in 9 days *Lincoln* and *A. E. Wright,* and in 15 days *Delépine,* were markedly alkaline; *Guy's* did not produce definite alkali till after a month's incubation.

B. Paratyphoid and food-poisoning group.

(α) Paratyphoid A.

1. *Schottmüller A :* original strain (1901).
2. *Brion and Kayser* (1902).

(β) Paratyphoid B.

Schottmüller B : original strain (1901).

(γ) Hog-cholera.

Aertryck: isolated from an outbreak of food-poisoning: from Prof. van Ermengem[1].

(δ) Gaertner.

1. *Gaertner L.I.P.M.:* old laboratory strain.
2. *Gaertner original A :* original strain from Prof. Gaertner.

For the most part all the members of this group B show identical cultural reactions. They are actively-motile "coli-like" organisms; no liquefaction of gelatine; indol none or in small or moderate amount;

acid and gas in	*dextrose*	dextrin[2]
	laevulose	mannite
	maltose[3]	*dulcite*
	galactose	*sorbite*
	arabinose	

but no apparent change in

lactose[4]	*salicin*
cane-sugar	amygdalin
raffinose[5]	inulin
erythrite	

[1] See van Ermengem in Kolle and Wassermann's *Handbuch*, 1903, vol. II. p. 657.

[2] The absence of dextrin fermentation in the paratyphoid B isolated by H. G. Wells and L. O. Scott (1904) is probably accounted for by the variation of the so-called "dextrin."

[3] Drigalski (1903, p. 421) found that *Aertryck* produced no change in maltose after repeated trials.

[4] H. Kayser (1904 *b*) describes paratyphoid B (but not A) as producing gas in lactose broth as does W. Korte (1903). A. Brion and H. Kayser (1902) in the original description of their organism say that it produces a small amount of gas in lactose broth.

[5] A. MacConkey (1905, p. 350) states that paratyphoid A and B produce acid and gas in

By their action on litmus-milk (or litmus-whey) they fall into two distinct classes: (1) paratyphoid A producing permanent[1] acidity without clotting; (2) paratyphoid B, hog-cholera and Gaertner producing an initial slight acidity which is succeeded after a very variable interval (2 to 14 days) by definite and increasing alkalinity.

The production of "indol" is variable[2]. Inasmuch, however, as small amounts are very frequently found in 5-day old broth cultures of paratyphoid B and hog-cholera, and very rarely (if at all) in similar cultures of Gaertner, a positive reaction may be of some value in diagnosis. A negative result is of less import.

The groups paratyphoid B, hog-cholera and Gaertner are then practically indistinguishable from one another by cultural methods. The work of H. Bruns and H. Kayser (1903), H. Trautmann (1903), H. de R. Morgan (1905), V. Porcile (1905) and others shows, however, that by their agglutination reactions they may be readily separated. It is also clear that *for the satisfactory identification of any of these organisms it is necessary to examine their reactions with sera prepared with known cultures*[3]. By these tests Gaertner is separated from paratyphoid B and from hog-cholera almost as clearly as is paratyphoid A from any of the other three. In most instances there is also not much difficulty in dividing paratyphoid B from hog-cholera. Indications are, however, not wanting that the line of separation between these two is

raffinose: he has been good enough to tell me that acidity of the medium (containing 0·5 p.c. sodium taurocholate and tinted with neutral red) to a greater or less degree was always noted: gas formation was sometimes present, but more frequently absent; when present it was only in slight amount. This I have failed to confirm with Kahlbaum's raffinose in litmus peptone-water as far as two strains of A and four of B are concerned. H. Conradi, W. v. Drigalski and G. Jürgens (1902) describe *Schottmüller B* and a strain of paratyphoid B isolated by themselves as without action on raffinose, and Drigalski (1903) found that no change is produced in this medium by paratyphoid B or Gaertner.

[1] On two occasions, both *Schottmüller A* and *Brion and Kayser* have, after about two months, become strongly alkaline. Libman (1902) found the same with Schottmüller's "Müller" (*i.e.* A) strain. The difference may after all be only one of temporal degree: this does not however introduce any practical difficulty.

[2] Most observers, *e.g.* Schottmüller (1901), Kranepuhl (1905), Kayser (1904 *b*), Korte (1903), have stated that no indol is produced by any of these organisms. Among others Morgan (1905), Libman (1902), Buxton (1902), Cushing (1900) and Savage (1905), note the production of small quantities.

[3] It may be hardly necessary to point out that in such experiments it is not enough to show that an unknown organism reacts at some arbitrary dilution with a known serum; the ultimate limits of agglutination should be determined for each serum with both the unknown organism and the strain and organism homologous with the serum. The diagnosis depends on the relative, not the absolute, value of the figures so obtained.

not always very well-defined. Thus Bruns and Kayser (1903) note that *B. breslaviensis* (which Morgan (1905) has shown to belong to the hog-cholera (*Aertryck*) group) reacts with *Schottmüller B* serum to a dilution limit not much less than that for its homologous organism; and Trautmann's (1903) tables make it clear that paratyphoid B is much closer to hog-cholera than is Gaertner to either. Similar results are shown in the following table, which gives the agglutination limits of the three organisms with nine sera:

	Serum	*Schottmüller B*	*Aertryck*	*Gaertner L.I.P.M.*
I.	Paratyphoid B; human	20,000	20,000	<100
II.	,, ,,	1,000	500	50
III.	,, ,,	5,000	1,000	200
IV.	*Schottmüller B*; rabbit	5,000	1,000	200
V.	,, ,,	5,000	200	200
VI.	*Aertryck*; rabbit	100	5,000	<50
VII.	,, ,,	2,000	2,000	20
VIII.	*Gaertner original A*; rabbit	100	50	20,000
IX.	,, ,,	20	20	5,000

H. Bonhoff (1904) goes further and concludes (on inadequate grounds) that paratyphoid B cannot be distinguished even from Gaertner. H. Smidt (1905) has published agglutination reactions showing that hog-cholera, paratyphoid B, and *B. typhi-murium* are very closely allied and far removed from paratyphoid A: from his results the specific distinction of *B. typhi-murium* (which according to Morgan (1905) belongs to the Gaertner group) and paratyphoid B would seem doubtful[1].

On the whole, the distinction between hog-cholera and paratyphoid B, though slender, seems to be real. The morbific relations to man are different, for while hog-cholera (*Aertryck, breslaviensis*, Hatton (Durham 1898 *b, c*), Düsseldorf (Trautmann 1903)) gives rise to a sudden acute illness (food-poisoning), paratyphoid B causes a disease with no clear clinical distinctions from enteric fever[2], and to which the sequelae of an attack of food-poisoning do not bear any close resemblance. Such a difference must needs carry considerable weight unless we hold with Trautmann (1904) that food-poisoning is only a very acute infection and intoxication (organisms + toxins) with the same essential cause as paratyphoid fever (organisms only).

[1] As a point of technique, it may be pointed out that these differences in agglutination are best brought out by sera from animals which have received many inoculations (see below p. 50, and Fox, 1905 *b*).

[2] See however Schottmüller (1904) on the rôle of these organisms in the causation of acute gastro-enteritis.

The protective results of vaccination also indicate a clear distinction between paratyphoid B and *Aertryck*. A series of guinea-pigs of about 200 gms. were inoculated subcutaneously with 0·1 c.c. of a 20 hours' culture of paratyphoid B (strain *Schottmüller B*) killed by heating at 60° for 30 minutes. Nine days later they were tested by intraperitoneal injection of living cultures of *Potts* and *Aertryck*; the latter alone was rapidly fatal[1]:

Potts			*Aertryck*		
Control	0·01 c.c.	dead <20 hrs.	Control	0·5 c.c.	dead <20 hrs.
,,	0·001 ,,	dead 28 hrs.	,,	0·1 ,,	lived
Vaccinated	0·01 ,,	lived	Vaccinated	2·0 ,,	dead <17 hrs.
,,	0·01 ,,	dead 25 days	,,	1·0 ,,	,, ,,
,,	0·002 ,,	dead 36 days	,,	1·0 ,,	,, ,,
,,	0·001 ,,	lived	,,	0·5 ,,	,, ,,

As was pointed out by Durham (1898 *b*) some time since, the quantitative measurement of the degree of relationship of two or more organisms by their interactions with various sera is not free from fallacy unless the bacilli concerned are closely related by other characteristics. Such results as are shown in the following table are very frequently

	Agglutination limits with			
Serum	*Guy's*	*Schott. B*	*Aertryck*	*Gaertner L.I.P.M*
Schott. B; rabbit	1,000	10,000	200	50
,, ,,	2,000	5,000	200	200
Gaertner L.I.P.M.; rabbit	1,000	20	20	5,000
,, *orig. A*; rabbit	2,000	100	50	20,000

obtained and might be taken as showing that paratyphoid B is more nearly related to typhoid than to hog-cholera. If, as seems necessary, cultural (biological) characters are to form the basis of our classification, such is of course by no means the case. The *B. typhosus* belongs to an entirely different group though it has affinities which will find available agglutinin in many different "immune" sera, or, perhaps better, it is an organism of high agglutinability.

[1] From other experiments it appeared that the specificity of such protection was not high; thus vaccination with Gaertner produced a distinct resistance to paratyphoid B. Sacquépée and Chevrel (*Comptes Rendus de la Société de Biologie*, 1905, vol. LIX. p. 598) have recently shown that the mutual heterologous protective powers of typhoid and paratyphoid A and B vaccinations are almost as great as the homologous.

III. *Occurrence of paratyphoid fever.*

During a period of eight months (February to October) specimens of blood from 347 patients were received at the Institute to be examined for the Widal reaction. The majority came from London and the adjacent districts. They were presumably in all cases derived from persons suffering from an illness bearing at least some resemblance to typhoid fever. Of these, 176 (51 per cent.) gave the serum reactions of typhoid, 166 were negative, and in five instances the diagnosis of paratyphoid infection was suggested as the result of the examination. Three of these were shown to be cases of infection with paratyphoid B, and the organism was isolated from the stools; the other two were probably of the same nature, though definite proof was not obtained. It has been the universal experience that infections with paratyphoid B are far commoner than with paratyphoid A: thus Pratt (1903) in his analysis of 84 cases found only 12 instances of A infection, and Zupnik (1905) had 21 cases of B and only one A. An abstract of the recorded A cases is given by Kayser (1904 *a*), since when practically all published cases have been B infections.

We thus find that about 3 per cent. of the cases of "typhoid" are in reality due to an organism belonging to a different group—a proportion small enough but sufficient to show that paratyphoid fever occurs as something more than a bacteriological curiosity in this country. If the observed proportion is approximately representative, it follows that, of the 3,000 odd cases of typhoid notified each year in London, nearly 100 should be separated as not due to Eberth's bacillus. The proportion is probably, if anything, too low: Schottmüller (1901) in Hamburg found five paratyphoids in 68 "typhoids" and Zupnik (1905) 7 per cent. in 300. Dr H. G. Wells of Chicago tells me that systematic blood-cultures show that about 10 per cent. of the "typhoids" in that city are really paratyphoids, and Kolle (1905) estimates the proportion in Germany at about the same.

The case mortality of the 84 cases of paratyphoids analysed by J. H. Pratt (1903) was 3·5 per cent., and H. Kayser (1903) estimates it at only 1 to 2 per cent. This is in marked contrast with the 17 per cent. found for typhoid in London, and it follows that an unusual prevalence of paratyphoid might render the figures for the fatality of typhoid materially inaccurate. The accurate diagnosis of the condition

is therefore of more than pathological interest; the favourable prognosis and the frequent absence of intestinal ulceration (Lucksch 1903, Wells and Scott 1904), should show a distinct reaction in the increased comfort of all concerned with such cases from the more strictly medical point of view.

Clinically the five present cases were all rather mild, and perhaps in two cases rather indefinite, typhoid. They all ended in recovery. It is somewhat curious that four of the cases occurred in boys of $1\frac{10}{12}$[1], 4, 8 and 13 years: the fifth (the precise nature of which has not been fully determined) was in a woman aged 45. They all came from separate localities—Epsom, Woolwich, Balham, Lancaster and Southfields (the infection in the last case having been clearly contracted in Dublin), and came under notice in March, June, July, August and September[2].

A detailed account of the bacteriological findings in these cases will be appropriately preceded by some general considerations with regard to the serum reactions in typhoid and paratyphoid infections and their use in diagnosis.

IV. *The secondary agglutinations of typhoid sera.*

It is well-known that typhoid immune serum may agglutinate other organisms as well as the *B. typhosus*, and that sera prepared by infection with these other organisms may agglutinate typhoid as well as their homologous bacilli. Such sera may be said to contain, besides the homologous, specific or primary agglutinin, heterologous, secondary or so-called "group" agglutinins. Some idea of the frequency of these subsidiary reactions may be gained from the figures obtained in the present series of tests on human sera, no serum being tested at a lower dilution than 1 : 20.

[1] G. B. Allaria (1903) records a case in a child of two years; the organism is, however, described as fermenting lactose and a good deal of doubt is thus thrown on the diagnosis. Count and Kirby (1904) describe a fatal case in a child of $4\frac{1}{2}$ months; the organism obtained did not ferment mannite, laevulose or maltose, and there seems to be no reason for regarding the case as one of paratyphoid infection. Feyfer and Kayser's (1902) case, aged two years, was paratyphoid only by inference.

[2] During the six months, February to August, 44 per cent. of the sera gave typhoid reactions and four cases of paratyphoid occurred to 98 cases of typhoid; in the next two months only one case of paratyphoid was found to 78 typhoid and 62 per cent. of the sera became "positive" as the prevalence of enteric increased.

Organism[1]	No. of sera tested	Found to be typhoid	Secondary agglutination positive
Typhoid: *Guy's*	347	176	—
Paratyphoid A: *Schott. A*	347	176	19 = 10·8 p.c.
,, *Brion and Kayser*	157	101	58 57·4
Paratyphoid B: *Schott. B*	347	176	55 31·2
Hog-cholera: *Aertryck*	156	101	31 30·7
Gaertner: *L.I.P.M.*	194	125	64 51·2

The details of 86 consecutive typhoid sera which were tested with all six organisms, and in which the agglutination limits for typhoid were fully worked out, are given in the next table. Sera giving no reaction at 1 in 20 are recorded as negative.

Agglutination limits with						
Guy's	*Schott. A*	*Brion and Kayser*	*Schott. B*	*Aertryck*	*Gaertner L.I.P.M.*	
20	—	—	—	—	—	21 cases
50	—	—	—	—	—	12 cases
	—	20	—	—	20	
	—	20	—	20	—	
	—	50	20	—	20	
100	—	20	—	—	—	
	—	20	—	—	20	
	—	20	—	—	50	
	—	20	—	—	50	
	—	20	20	—	20	
	—	100	20	—	20	
	—	20	20	20	20	
200	—	—	—	—	20	
	—	50	—	—	20	
	—	50	—	—	20	
	—	50	—	—	50	
	—	—	20	—	100	
	—	100	20	—	50	
500	—	—	—	—	—	
	—	50	—	—	—	
	—	20	—	—	20	
	—	50	—	—	50	
	—	50	50	—	200	
	—	200	50	20	200	
	20	50	100	20	20	

[1] The sera were at first tested only with typhoid, *Schottmüller A* and *Schottmüller B*: *Gaertner* and subsequently *Aertryck* were added, and *Brion* and *Kayser* used as well as *Schottmüller A* when the very low inherent agglutinability of this latter strain became known by tests with artificial homologous sera.

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	Agglutination limits with				
Guy's	*Schott. A*	*Brion and Kayser*	*Schott. B*	*Aertryck*	*Gaertner L.I.P.M.*
1,000	—	200	—	—	200
	—	100	50	—	500
	—	200	100	—	500
	—	50	20	20	50
	—	50	50	20	50
	—	50	20	20	200
	—	50	20	20	200
	—	50	500	50	100
	20	50	100	20	100
	20	500	20	20	500
	20	500	50	50	500
2,000	—	—	—	—	—
	—	20	—	—	200
	—	100	50	20	20
	—	500	20	20	1,000
	—	1,000	50	50	100
	20	200	200	100	200
5,000	—	100	—	—	200
	—	500	2,000	500	—
	—	200	100	100	200
	—	100	100	50	200
	—	100	100	100	200
	20	100	50	50	200
	50	100	100	100	1,000
	50	2,000	200	200	500
10,000	—	—	100	200	1,000
	—	200	20	20	200
	—	200	200	200	500
	—	200	200	500	5,000
	50	200	200	200	500

Of this series, 51 (59 per cent.) showed secondary agglutinations while 35 (41 per cent.) reacted with typhoid only; 55 per cent. reacted with *Gaertner L.I.P.M.* or *Brion and Kayser*, 41 per cent. with *Schottmüller B*, 33 per cent. with *Aertryck*, and 12 per cent. with *Schottmüller A*. The titres of these coagglutinations and the combinations in which they occurred are summarized in the next tables.

	Number of cases which reached an agglutination limit of						
	20	50	100	200	500	1000 and over	Total
Schottmüller A	7	3	—	—	—	—	10
Brion and Kayser	10	14	9	9	4	2	48
Schottmüller B	12	8	8	5	1	1	35
Aertryck	13	5	4	4	2	—	28
Gaertner L.I.P.M.	12	7	4	13	7	4	47

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Typhoid only	35
Typhoid + Brion and Kayser + Schott. B + Aertryck + Gaertner	16
Typhoid + Brion and Kayser + Gaertner	12
Typhoid + Schott. A + Brion and Kayser + Schott. B + Aertryck + Gaertner	10
Typhoid + Brion and Kayser + Schott. B + Gaertner	7
Typhoid + Brion and Kayser	2
Typhoid + Gaertner	1
Typhoid + Schott. B + Aertryck + Gaertner	1
Typhoid + Schott. B + Gaertner	1
Typhoid + Brion and Kayser + Aertryck	1
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The fact that typhoid sera may also agglutinate Gaertner bacilli seems to have been first noted by Grüber and Durham (1896), and subsequently developed by Durham (1898 *a*, *b*) who elaborated the correct interpretation of the phenomenon (1901). Pioneer observations were also made by Lorrain Smith and Tennant (1899) who found that, of 123 cases in which the blood of clinical typhoids reacted with typhoid bacilli, 24 per cent. reacted also with Gaertner and 11 per cent. with *Aertryck*[1]. Klein (1903) noted that most typhoid sera would also agglutinate Gaertner to some extent, and Fox (1905 *b*) found Gaertner reactions in 87 per cent. of 94 cases. Zupnik and Poser (1903) found 89 per cent. of 64 typhoid sera to react with paratyphoid B and 40 per cent. with paratyphoid A; Korte and Steinberg (1905) had 56 per cent. and 53 per cent. respectively in 70 cases, and Manteufel (1905) 62 per cent. and 60 per cent. in 85 cases. Exact comparison between these and the present figures is not possible owing to differences in minimal dilutions and standards of reactions.

There is thus considerable variation in the presence, degree, and kind of subsidiary agglutinations in different typhoid sera. The most obvious factor in this variation as regards presence and degree is the typhoid-strength; from the figures already given it appears clear that the more typhoid agglutinin is present, the more subsidiary agglutination is likely to be found. Thus of the 36 sera with a typhoid-strength of less than 1 : 100 only 8 per cent. show coagglutination, while of 50 which rise above that figure 96 per cent. agglutinate also other bacilli. The table shows, however, that marked exceptions to this rule may occur; thus one serum reacting at 1 : 2,000 with typhoid failed to agglutinate

[1] The "*B. enteritidis Hatton*" of Smith and Tennant appears to be a hog-cholera (*Aertryck*) organism. Durham (1898 *b*) found that agglutination tests placed the organism he isolated from the Hatton outbreak with "Günther" (1897) rather than with Gaertner's original strain, and Morgan (1905) has shown that "Günther" is an Aertryck organism.

any of the other organisms at 1 : 20. Others have obtained similar results: thus Manteufel (1905), examining 85 typhoid sera with typhoid, paratyphoid A and paratyphoid B, found coagglutinations present in 56 per cent. of those with a typhoid-strength of 200 or less, and in 78 per cent. of those stronger than this. The great variability in the coagglutination associated with the same typhoid-strength in different sera and in the same serum at different times is, however, sufficient to indicate that a serum does not possess this property of reacting with others than the homologous organism in virtue of its homologous strength alone. It should not be held that typhoid agglutinin is able to produce agglutination of, *e.g.* Gaertner bacilli, if it be present in sufficiently high concentration, but rather that most typhoid agglutinins are possessed of combining groups for other bacilli in quantities which are very variable but (nearly always) far less than for typhoid. It is only when the amount of typhoid agglutinin rises to a fair height that these subsidiary groups become sufficiently numerous to be appreciable by the ordinary methods of examination. That secondary agglutinations are not a function of the strength of the primary agglutinin is also demonstrated by absorption tests: it is found that the secondary agglutination may be entirely removed by the homologous subsidiary organism while the strength of the primary agglutinin is still left above the limits which are commonly associated with the presence of coagglutinations.

The next table of 65 human sera shows that, as the titre for typhoid increases, so the *relative* amount of subsidiary agglutinins diminishes:

Typhoid titre	Number of cases	Average total absolute secondary agglutinins	= per cent. of typhoid agglutinin
50	15	11	23
100	7	69	69
200	6	92	46
500	7	167	33
1,000	11	533	53
2,000	6	645	32
5,000	8	1,196	24
10,000	5	1,978	20

In a general way the increase of typhoid agglutinin accompanies the further progress of the earlier part of the attack of typhoid fever; there is then a tendency to relative purification as the infection persists. This is shown by the following agglutinative histories of four rabbits, which also illustrate the general variability which occurs:

Rabbit I: typhoid *A. E. Wright.*

Time days	Number of inoculations	Absolute titre for typhoid	Total[1] absolute secondary agglutinins	Relative (typhoid=100) titre for				
				Schott. A	*Schott. B*	*Aertryck*	*Gaertner*	Total
7	2	50	4	4	0	0	4	8
12	2	1,000	160	0	1	5	10	16
21	3	2,000	290	1	1	2·5	10	14·5
56	4	2,000	200	0	0	0	10	10

Rabbit III: typhoid *Lincoln.*

7	2	200	140	1	50	10	10	71
12	2	1,000	160	0	5	1	10	16
21	3	2,000	120	0	2·5	2·5	1	6
56	4	10,000	100	0	1	0	0	1

Rabbit II: typhoid *A. E. Wright.*

Absolute titre for typhoid	Total absolute secondary agglutinins	Relative (typhoid=100) titre for				
		Schott. A	*Schott. B*	*Aertryck*	*Gaertner*	Total
200	22	0	5	1	5	11
500	40	0	4	2	2	8
2,000	140	0	1	1	5	7
5,000	70	0	0	0·4	1	1·4

Rabbit IV: typhoid *Lincoln.*

500	160	2	2	10	20	34
1,000	400	1	10	10	20	41
2,000	1,100	1	2·5	2·5	50	56
		(dead)				

[1] *I.e.* total for those organisms which were used: other secondary agglutinins (*e.g.* for *B. coli*, *B. dysenteriae*) were doubtless present. The minimal dilution in these observations was 1 : 2.

As far then as any temporal relations can be laid down as to the occurrence of secondary agglutinins, it would appear that they are apt to be especially prominent in the early stages of reaction to infection; this absolute and relative abundance is replaced by a stage of relative decrease and absolute increase, which is in turn succeeded by a tendency towards absolute decrease. Fox (1905 *a*, *b*) has especially called attention to the occurrence of Gaertner agglutinins in both human and rabbit typhoid sera before the appearance of the typhoid reaction, and finds that the agglutinative purity of the serum increases as the disease progresses. Park and Collins (1904, p. 505) note this, and also call attention to a marked decrease of purity which may occur after prolonged artificial immunisation.

Other factors are race of infecting organism, and species and individual of infected animal. The former is illustrated by the last table: in the first three weeks, the two typhoid *Lincoln* rabbits produced more than three times the total relative quantity of secondary agglutinins as those inoculated with typhoid *A. E. Wright*, the primary agglutinin being about the same in all four animals. The same animals illustrate the individual variation. With regard to the influence of species, Klein (1904, p. 478) has pointed out that sera of mixed agglutinations are much more readily induced in the rabbit than in the guinea-pig by inoculation of typhoid or Gaertner.

Little need be said as to the kind of secondary agglutinin developed under varying circumstances. It is clear that those for paratyphoid A (*Brion and Kayser*) and Gaertner are the most frequent and generally the stronger: in the series of human sera already given (p. 45), Gaertner is preeminent 19 times, *Brion and Kayser* 9 times, and the two together 13 times. The other type of secondary agglutination among the organisms concerned is much less frequent, and paratyphoid B is in excess but three times. Nothing is clear as to the circumstances which influence this.

Paratyphoid and Gaertner immune sera contain relatively less secondary agglutinins. As the results of typhoid sera suggest, they all react with typhoid freely and usually to a much greater degree than with the heterologous members of their own group. In other words typhoid bacilli have many more combining groups of low specific affinities than have the other varieties of organisms under consideration.

V. *The serum diagnosis of paratyphoid infection.*

The absolute bacteriological diagnosis of paratyphoid fever must involve the isolation of one of the two specific organisms from the stools, urine, pus or, preferably, the blood of the patient, followed by the satisfactory identification of the bacillus by cultural and agglutination tests. Conradi (1904) has shown that paratyphoid, like typhoid, bacilli may occur in the stools of healthy contacts: unless therefore the organism has been found in some part of the body more truly internal than the alimentary tract, the presence also of a state of specific infection must be shown by the serum reactions of the blood. Irregular local paratyphoid affections seem to be at least as common as those caused by typhoid bacilli, appearing either as sequelae to a typhoid-like illness or arising apparently independently of any general infection. In the latter category fall, for instance, the cases recorded by Widal and Nobécourt (1897) who found their "paracolibacille" (= paratyphoid B) in a thyroid abscess, and by Blumenthal (1904) who isolated paratyphoid A from the gall-bladder. Netter and Ribadeau-Dumas (1905) have also recently observed that the sera of a number of cases of acute jaundice agglutinate paratyphoid A (*Brion and Kayser*) to a limit considerably beyond that for typhoid; they suggest that such cases are in reality an "angiocholicystite paratyphique." As examples of the discovery of paratyphoid in local "post-typhoid" suppurations, we may recall the recovery of paratyphoid B from an osteomyelitis by Achard and Bensaude (1896) and Cushing (1900), and from an orchitis as well as from the gall-bladder four years after an attack of "typhoid" by Pratt (1903). In these local infections, the serum reactions are commonly not very well-developed; their rather common occurrence must however be considered in the interpretation of the phenomena observed in the examination of the blood.

Considerations of time and opportunity however must often render complete examinations impossible. It is therefore desirable to consider how far a diagnosis may be formed upon the serum reactions alone.

(*a*) *Agglutinations.* The diagnosis cannot be made on a demonstration that the serum of a patient suffering from an illness resembling typhoid fever fails to agglutinate the *B. typhosus* even in low dilutions (*e.g.* Mackie 1905). Still less is the diagnosis of paratyphoid infection warranted by the bare fact that agglutination is observed with paratyphoid or similar organisms: this is a common property of typhoid and

other sera. Whether a reasonably probable diagnosis can be made after a complete determination of the ultimate limits of agglutination with both paratyphoid and typhoid bacilli is an important practical matter on which opinion is much divided. On the one hand Jürgens (1903, 1904), Drigalski (1904), Grünberg and Rolly (1905), Pratt (1905), Fox (1905 *a*, *b*), Conradi (1904) and Kayser (1904 *a*) are, among other observers, very sceptical as to the value of agglutinations in the differential diagnosis of typhoid and paratyphoid, while of recent authors Korte and Steinberg (1905), Manteufel (1904) and Zupnik (1905) believe that the method gives on the whole accurate results. Pratt (1903), for example, records a case of "typhoid" where the serum reacted with paratyphoid B at 1 : 200 and gave no reaction with typhoid at 1 : 10; blood cultures gave a pure culture of *B. typhosus*. Fox (1905 *a*) obtained *B. typhosus* from the blood of a patient whose serum agglutinated paratyphoid A at higher dilutions than typhoid throughout the illness; the difference was most marked during a relapse. The same author (1905 *b*) notes that some reaction with Gaertner was present in 82 of 94 typhoid sera, and that in about a dozen cases this secondary agglutination appeared earlier in the disease than, or exceeded the limit of, the typhoid reaction. The results of Grünberg and Rolly (1905) are even more remarkable: of 40 cases of undoubted clinical typhoid, 32 gave *B. typhosus* on blood culture; coagglutinations were present in 28 and in 14 the reactions with paratyphoid A or Gaertner occurred at higher dilutions than with typhoid. Such results are in strong contrast with those of Korte and Steinberg (1905) and Manteufel (1905) who examined respectively 70 and 85 typhoid sera with *Schottmüller A* and *B* and found no case where the agglutination limits for these surpassed the limit for typhoid. In the present series of 176 typhoid sera, the secondary agglutinations were never observed to be in excess of the primary reaction: in two instances the limits ascertained for typhoid and paratyphoid A (*Brion and Kayser*) were the same, though it should be noted that the limits reached were only 1 : 50 and 1 : 100. A later sample of serum from one of these cases gave typhoid = 1 : 100, *Brion and Kayser* = < 1 : 20: the other could not be further examined.

In a valuable contribution dealing with 300 cases of typhoid, Zupnik (1905) records only the following instances of irregularity in the agglutination results:

B. typhosus isolated from	Agglutination limits for		
	Typhoid	*Schottmüller B*	*Brion and Kayser*
Spleen	80	200	0
Blood	0 (<40)	80	0[1]
Faeces	1,600	120	2,000
Spleen	80	0	80
Urine	200	200	0
Blood	200	40	200[1]

He points out that equality or inferiority of typhoid agglutinin is likely to be less frequent the greater the number of races of typhoid used for the tests; further that, as far at any rate as paratyphoid B is concerned, *approximate or complete equality of typhoid and paratyphoid agglutination limits indicates a typhoid infection.* In 21 cases of infection with paratyphoid B, the least difference between the paratyphoid and typhoid agglutination was in a case where the ratio of the two was 6·2 : 1, the average ratio being about 240 : 1. In none of the recorded cases has the superiority of paratyphoid over typhoid in a typhoid serum been of the same order of magnitude. In this connection the high absolute figures often obtained with paratyphoid B sera may be noted: generally these are far beyond the values obtained for typhoid in typhoid sera. Thus Savage (1905, p. 344) notes a reaction of 1 : 70,000, my own cases vary from 1 : 5,000 to 1 : > 20,000, and of Zupnik's (1905, p. 1751) 21 patients only one was less than 1 : 1,000, nine exceeded 1 : 10,000 and three reached the remarkable figures of 1 : 96,000, 1 : 128,000 and 1 : 140,000.

The opinion that the results of agglutination experiments are unreliable is to some extent founded on errors of technique. Thus Drigalski (1904) found that in 9·3 per cent. of 257 cases the agglutination of paratyphoid B was equal to, and in 10·1 per cent. more than, that of typhoid. The conclusion that the method is unreliable is altogether invalidated by the fact that the observations were only made at dilutions of 1 : 50 and 1 : 100, no attempt being made to ascertain the *ultimate limits* of agglutination for the two organisms. Attempts to measure the amount of agglutinin by the apparent completeness of the reaction at a low dilution result only in an increase of the errors which are inherent to the method under the most favourable circumstances.

Other sources of error may occur in the organisms used for agglutination. Three somewhat striking examples of the failure of an animal serum to identify its homologous bacillus have been met with in the course of the present investigation. The first illustrates the importance

[1] Later examinations of these cases showed a higher limit for typhoid.

of ascertaining the conditions of growth of the test cultures best suited for agglutination experiments.

Rabbit: inoculated with *Lincoln* typhoid four times in a period of 29 days: agglutination limits of serum:

Conditions of growth of test culture	Room 17 hrs.	36° 17 hrs.	43° 17 hrs.	43° 3¼ hrs.
Typhoid *Guy's*	2,000	2,000	2,000	2,000
,, *Lincoln*[1]	1,000	100	100	<50
Schottmüller A	—	20	—	—
,, *B*	—	50	—	—
Aertryck	—	50	—	—
Gaertner L.I.P.M.	—	500	—	—

The second instance illustrates the difficulties which are raised by an infection with an organism which, though agglutinogenic, is relatively non-agglutinable. Such was the condition of the strain of *Schottmüller A* used in these experiments. Two rabbits were inoculated repeatedly with this organism: their sera gave the following results:

	Agglutination limits with					
	Typhoid *Guy's*	*Schott. A*	*Brion and Kayser*	*Schott. B*	*Aertryck*	*Gaertner L.I.P.M.*
Rabbit A: 27. vi. '05	1,000	100	—	20	20	200
Rabbit B: 24. vii. '05	100	10	—	10	<10	10
1. viii. '05	200	20	<20	<20	20	20
15. viii. '05	500	100	2,000	500	500	500

In the first case the use of a culture grown at about 17° instead of 36° brought the agglutination results in correspondence with the known origin of the serum. In the second instance however no such procedures produced any material modification in the agglutinability of the homologous organism; the use of another strain (*Brion and Kayser*) of the same bacillus was here necessary.

The third example of such errors is of the opposite nature. The culture of *Brion and Kayser* in our possession, when emulsified in any mixture of normal or immune serum at any dilution, shows a remarkable tendency to spontaneous sedimentation. So much so, that, after two hours in the hot incubator, the macroscopic agglutination is in nearly all cases practically complete, though microscopically in the hanging drop there may be no reaction at all. The use of such an organism in the macroscopic way would lead to the most erroneous conclusions, and it is perhaps not insignificant that many of the results, which are

[1] It is possible that this phenomenon is a partial explanation of the lack of agglutinability commonly ascribed to recently-isolated typhoid strains. The old laboratory strain (*Guy's*) gave the same results under all conditions.

considered to condemn the use of agglutination phenomena for the differential diagnosis of typhoid and paratyphoid infections, have been obtained by the macroscopic method.

Korte and Steinberg (1905) have called attention to another possible source of fallacy arising out of the "zone of inhibited agglutination." In low dilutions, a typhoid serum may fail to agglutinate typhoid bacilli, giving at the same time a good reaction with paratyphoid; at higher dilutions the typhoid reaction appears, and ultimately far surpasses that with paratyphoid. It is interesting to note that the authors found this phenomenon to be materially misleading only when the macroscopic method was used; microscopical controls revealed the real nature of the serum without much difficulty.

On the whole then, it would appear that an accurate determination of the ultimate limits of agglutination, preferably by the microscopic method, with organisms of known agglutinable capacity, and with cultures grown under predetermined optimum conditions, will, at any rate in the great majority of cases, give accurate information as to the nature of a typhoid or paratyphoid serum. At the same time it must be allowed that anomalous results are occasionally obtained, especially in the early stages of infection, and a positive diagnosis of paratyphoid should not be lightly made since doubtful cases are usually typhoid.

(*b*) *Absorption tests and mixed infections.*

The determination of the agglutination values of the different organisms reacting with any given serum will not, however, exclude the presence of a double or multiple infection. Such mixed infections are necessarily uncommon in so far as typhoid, paratyphoid and similar organisms are concerned. The incidence of clinical typhoid fever in London varies but little from 0·07 per cent.; paratyphoid fever can hardly be more frequent than 0·005 per cent.: the coincidence of the two diseases should therefore be of almost infinite rarity. There is however little reason to doubt that paratyphoid is disseminated by the same excretory contamination, direct and indirect, which is responsible for the spread of typhoid. This similarity must considerably reduce the mathematical improbability of their coincidence. Typhoid and paratyphoid organisms have in fact been isolated from the same person by several observers (Conradi 1904, Kayser 1904 *a*: see also Savage 1905, p. 349), though such instances are in part no doubt cases of para-

typhoid infection in an individual harbouring, but not in a state of infection by, typhoid organisms (or *vice versâ*).

For the diagnosis of mixed infections, absorption experiments ("Castellani's test") are of the first importance. Castellani (1902) found that excess of typhoid bacilli would remove not only the primary (homologous) typhoid, but also the secondary (heterologous) *coli*, agglutinins from the serum of a rabbit inoculated with typhoid, while from a serum giving similar agglutinations (at any rate qualitatively) but elaborated in response to infection with *B. coli* as well as *B. typhosus*, neither typhoid nor *coli* bacilli alone, but only both together, simultaneously or successively, would remove the whole of the agglutinin.

The accuracy and value of the test is generally acknowledged; some illustrative examples of single and double infections are given here. The technique used has been already explained (p. 37).

Nature of serum	Dilution[1]	Absorbed with	Agglutination (after absorption) with: Typhoid *Guy's*	*Brion and Kayser*	*Schott. B*	*Aertryck*	*Gaertner L.I.P.M.*	*Schott. A*
I. Typhoid: human	1 : 10	Original titre	2,000	1,000	50	50	500	50
		Typhoid Guy's	+++	0	0	0	+++	—
			0	0	0	0	0	—
		Brion and Kayser[2]	+++	++	0	0	+++	—
			+++	0	0	0	+++	—
		Schott. B	+++	++	0	0	++	—
			+++	0	0	0	++	—
		B. coli communis[3]	+++	++	+++	++	+++	—
II. Paratyphoid B: human: case "Barkley" 31. vii. '05: see also p. 64.	1 : 10	Original titre	200	500	>5,000	1,000	200	50
		Typhoid Guy's	0	+++	+++	+++	+	—
		Brion and Kayser	++	+++	+++	+++	+++	—
			++	0	+++	+++	+++	—
		Schott. B	0	0	+++	+++	0	—
			0	0	+++	+++	0	—
			0	0	0	0	0	—
		Aertryck	0	0	+++	0	0	—
		Gaertner L.I.P.M.	0	+++	+++	+++	0	—
		B. coli communis	+++	+++	+++	+++	+++	—
		Guy's + Brion and Kayser + Aertryck + Gaertner	0	0	+++	0	0	—

[1] The agglutinations were observed at twice this figure.

[2] These two series of figures under one heading indicate the results of successive additions of absorbing bacilli and show to some extent the order of removal of the different secondary agglutinations. Thus after the first extraction of the serum with *Brion and Kayser* the remainder agglutinated *Guy's*, *Brion and Kayser* and *Gaertner*; after a second extraction, *Guy's* and *Gaertner* still reacted and the *Brion and Kayser* agglutination failed (*i.e.* the end point of this absorption was reached).

[3] The original Escherich strain.

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Nature of serum	Dilution	Absorbed with	Agglutination (after absorption) with: Typhoid Guy's	Brion and Kayser	Schott. B	Aertryck	Gaertner L.I.P.M.	Schott. A
III. Gaertner original A: rabbit	1 : 25	Original titre	1,000	200	20	<20	5,000	50
		Typhoid Guy's	0	0	0	—	+ + +	—
		Brion and Kayser	+ +	0	0	—	+ + +	—
		Schott. B.	+ + +	0	0	—	+ + +	—
		Gaertner L.I.P.M.	0	0	0	—	0	—
		Aertryck	+ + +	0	0	—	+ + +	—
		B. coli	+ + +	+	0	—	+ + +	—
IV. Aertryck: rabbit	1 : 50	Original titre	200	500	2,000	2,000	20	—
		Typhoid Guy's	0	+ + +	+ + +	+ + +	—	—
		Brion and Kayser	0	0	+ + +	+ + +	—	—
		Schott. B	0	+	0	+ + +	—	—
		Aertryck	0	0	0	0	—	—
V. Typhoid *Delépine* + Schottmüller B: rabbit	1 : 50	Original titre	1,000	2,000	5,000	200	<20	—
		Typhoid Guy's	0	0	+ + +	+ +	—	—
		Brion and Kayser	+ + +	0	+ + +	+ +	—	—
		Schott. B	+ + +	0	0	0	—	—
		Typhoid + Schott. B	0	0	0	0	—	—
		Schott. B + Brion and Kayser	+ +	0	0	0	—	—
		Typhoid + Brion and Kayser	0	0	+ + +	+ +	—	—
VI. Typhoid *A. E. Wright* + Brion and Kayser: rabbit	1 : 25	Original titre	5,000	1,000	100	20	200	—
		Typhoid Guy's	0	+ + +	—	—	0	—
		Brion and Kayser	+ + +	0	—	—	+ + +	—
		Gaertner L.I.P.M.	+ + +	+ + +	—	—	0	—
		Gaertner + typhoid	0	+ + +	—	—	0	—
		Gaertner + Brion and Kayser	+ +	0	—	—	0	—
		Typhoid + Brion and Kayser	0	0	—	—	0	—
VII. Schottmüller A: rabbit	1 : 25	Original titre	500	2,000	500	500	500	100
		Typhoid Guy's	0	+ + +	+ + +	+ + +	0	0
		Schott. A	0	0	+ +	+ +	—	—
		Brion and Kayser	0	0	+ + +	+ +	—	0
		Schott. B	0	+ + +	0	0	—	0
		Aertryck	0	+ + +	0	0	—	+

These figures require little comment. Example IV illustrates the diagnostic import of absorption when the agglutination values for the homologous and a heterologous organism are approximately identical. Example VII shows the irregular results obtained in dealing with an organism of bizarre agglutinative character (see p. 54). Apart from

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this, no instance has been noted in which the absorption results have been demonstrably misleading, though indications have been obtained which suggest that Castellani's rule may not always hold good if the subsidiary organisms are widely heterologous to the reacting serum (see below p. 68). Opportunity has however been lacking for experimentation with sera of irregular agglutination. In most cases the results of absorption follow those of agglutination, and the two phenomena are essentially the same. They differ however in that absorption is conditioned by the total quantity of available combinations, while the immediate ocular demonstration of this reaction depends on the quantity of such combinations in immediate connection with the bacillary bodies. The occurrence and varying relative abundance of receptors free in the circumjacent fluid[1] are important factors over which we have imperfect control: they probably account for some of the irregularities observed.

Jürgens (1903, 1904) has described the absorption phenomena with typhoid sera of irregular agglutination limits. A typical example gave

	Agglutination limits with	
	Typhoid	Paratyphoid B
Normal	50	800
Absorbed with typhoid	0	300
,, ,, para. B	50	100

and is interpreted as being indicative of typhoid infection on account of the failure of paratyphoid B bacilli to remove any of the typhoid agglutinin. It is however difficult to understand how this could happen if the typhoid bacilli were able to remove more than half the paratyphoid agglutinin. My own experiments, as far as they cover the same ground, are not in accordance with this observation. They show that the primary agglutinin is absorbed by saturation with the bacilli of the secondary agglutinins, and the secondary agglutinins by the bacilli of one another, in amounts which are in a general way proportional to the quantities of secondary agglutinin present, though some allowance has to be made for the varying degree to which the combining groups of the different bacilli are mutually available[2]. For example:

[1] See M. Neisser and K. Shiga (*Deutsche medizinische Wochenschrift*, 1903, p. 61) and B. H. Buxton and J. C. Vaughan, Jr. (*Journal of Medical Research*, 1904, vol. XII. p. 138).

[2] Without entering at length on a complex subject, it may be briefly stated that these mutually combining groups appear from absorption experiments to be arranged in the same way as would be expected from the agglutination reactions. Thus *Brion and Kayser*, and *Aertryck* are more combinable, both mutually and with typhoid, than with paratyphoid B or Aertryck; this reproduces the arrangement of the frequency and intensity of the secondary agglutinins in typhoid and paratyphoid sera (p. 45). The hypothesis of Durham (1901) affords the best terms in which to express the phenomena.

Rabbit: typhoid (*A. E. Wright*) immune serum.

	Agglutination limits with			
	Typhoid *Guy's*	*Schott. A*	*Schott. B*	*Gaertner L.I.P.M.*
Before absorption	2,000	0	20	200
Absorbed with Typhoid *Guy's*	500	0	0	0
,, ,, *Schott. A*	2,000	0	20	—
,, ,, *Schott. B*	2,000	0	0	—
,, ,, *Gaertner L.I.P.M.*	500	0	20	0

Park and Collins (1904, p. 498) have called attention to the fact that absorption experiments may give erroneous results in mixed infections unless the bacteria concerned have been already suggested by agglutination or other phenomena. The paratyphoid A (*Brion and Kayser*) agglutinin, for example, found in the serum (number V above) of a typhoid infection may be completely removed by saturation with typhoid bacilli, and may be due in large part or altogether to an unsuspected concurrent infection with paratyphoid B. Its true nature would not appear unless the paratyphoid B infection had been already suggested and absorption tests with paratyphoid B bacilli made in consequence. "The absorption method simply proves, therefore, that when one variety of bacteria removes all agglutinins for a second, that the agglutinins under question were not produced by that second variety[1]."

(*c*) *Variation in type of agglutination.*

Attention has already been drawn to the fact that the "clumps" formed by Gaertner, paratyphoid B or Aertryck bacilli with homologous sera are relatively loose aggregations and of very different appearance to the dense masses seen in the reaction of typhoid bacilli with typhoid serum. The same form of reaction is commonly seen when any one of these three organisms reacts with a serum homologous with either of the other two. It is of some theoretical interest and of considerable practical diagnostic value to note that, when typhoid serum reacts with these organisms in a heterologous way, the clumps follow the typhoid type and appear as tight masses. The same, but to a less degree, is true for paratyphoid A: thus, with a *Brion and Kayser* rabbit serum, *Brion and Kayser* often forms loose masses (though frequently tight clumps) while paratyphoid B, Gaertner and *Aertryck* form typhoid-like clumps, as does *Brion and Kayser* in typhoid serum. To typhoid bacilli

[1] These considerations render the diagnosis of the much discussed *coli* infections in typhoid fever and similar conditions rather complicated owing to the marked specificity of the agglutinins of different races of *B. coli* (van Everen 1905).

the phenomenon does not apply; the clumps in homologous and heterologous sera are not markedly dissimilar.

These differences are entirely independent of the concentration of the serum and, as far as the cultures at my disposal are concerned, of the race of organism. Their common usefulness is found in the routine examination of typhoid sera in low dilutions where the typhoid-like clumps of the Gaertner reaction which is so often present suggest at once that this is a secondary, rather than a primary, agglutination.

(*d*) *The thermostability of paratyphoid agglutinins.*

It is well-known that agglutinins are relatively thermostabile, *i.e.* they are not rendered inactive by heating at 60° C. for 30 minutes. The agglutinins for Gaertner and paratyphoid have been found to be relatively more heat-resistant than those for typhoid. In these experiments the sera under comparison were mixed and then heated successively at 65°, 70°, 75°, etc. in a water-bath for 30 minutes at each temperature. A typical observation on a mixture of four immune rabbit sera gave the following agglutination limits:

	Typhoid *Guy's*	*Brion and Kayser*	*Schott. B*	*Gaertner L.I.P.M.*
Before heating	10,000	10,000	500	500
Heated at 70°	5,000	10,000	500	500
,, ,, 75°	<20	5,000	200	200
,, ,, 80°	—	500	<20	20
,, ,, 85°	—	<20	—	<20

The next table gives the average results of six such experiments with sera of various concentrations; the numbers represent the agglutination limits after heating expressed as percentages of the limits found in the unheated sera:

	Absolute limits before heating					
	Max.	Min.	Unheated	70°	75°	80°
Typhoid	10,000	500	100	22	0	0
Gaertner	20,000	500	100	83	32	6
Paratyphoid B	5,000	500	100	55	30	0
Brion and Kayser	10,000	1,000	100	63	45	4½

In such experiments it seems to be immaterial (within the limits of error of the method) whether the serum is slightly (1 : 10) diluted and then heated, the necessary further dilutions being made subsequently, or whether the serum is fully diluted before heating. In some instances the disappearance of agglutinin seems to be somewhat greater in the former circumstances, in others in the latter. The results are, however,

naturally different if the serum tested at one temperature has been previously exposed to a lower temperature, so long, that is, as this latter be above the lower limit at which destruction[1] begins to be manifest in periods of time comparable to 30 minutes. It should be clearly recognised that the destruction by heat of these properties of serum is not instantaneous but a gradual process, occupying at temperatures in the neighbourhood of 60° periods of time such that the conventional half-hour can in no way be regarded as infinite. It is a function of time as well as of temperature, so that the absolute amount destroyed at any given temperature is conditioned by the amount originally present if the time-limit falls materially short of that period in which the rate of destruction by that temperature falls almost to zero. The apparent effect of any heating is then distinctly different with sera of different strengths. If, for example, a certain combination of time and temperature reduces the agglutination limit by 95 per cent., the residues of two sera of titres 5000 and 100 will be 250 and 5 respectively. In the former case the agglutinin is manifestly only reduced, in the latter it is commonly spoken of as being destroyed. There are, however, certain temperature limits below which the times necessary to destroy any considerable quantity of agglutinin are very long relatively to 30 minutes. Using such a short period of heating, it is then perhaps permissible to speak of the destruction beginning and ending at certain points. From the results here obtained it would appear that this destruction of typhoid agglutinin commences at about 58° and is complete at about 75°, the corresponding figures for Gaertner agglutinin being 65 + ° and 83°. The coarse methods of measurement used tend however to obscure the earlier phases and to place the apparent point of commencing destruction somewhat higher in ordinary practice. It follows that 30 minutes at 65° *plus* 30 minutes at 70° will affect a Gaertner agglutinin to much the same degree as one heating at 70°: the latter will however have the smaller effect on a typhoid agglutinin.

The practical application to diagnosis of the differences in thermostability is rendered somewhat difficult by the preponderating influence of the quantities of agglutinin subjected to examination, as well as by the fact that different sera yield rather varying results even when allowance is made for their different concentrations[2]. It lies

[1] A convenient mode of expression and perhaps justifiable: *qua* the phenomenon of agglutination, agglutinins are destroyed by conversion into agglutinoids.

[2] It is important in practice to see that the serum to be heated is free from red cells and haemoglobin. Heating these is liable to produce a very fine precipitate which cannot be

however in the observation that *any Gaertner or paratyphoid agglutinin found in a typhoid-immune serum disappears at a lower temperature than the primary typhoid agglutinin.* Differences of concentration may be the whole explanation of the abolition of the Gaertner reaction at a lower, rather than at the same, temperature, though it would appear that secondary agglutinins generally are distinctly less thermostabile than the primary agglutinins. Thus a Gaertner-immune serum giving Gaertner = 20,000 and typhoid = 5,000 showed, after heating for 30 minutes at 60°, Gaertner = 20,000 and typhoid = 100, while typhoid-immune sera of comparable strength under similar circumstances show a loss of less than 50 instead of more than 90 per cent.

It is perhaps germane to this subject to recall the fact that the toxins of Gaertner and paratyphoid are notoriously heat-resistant. A remarkable elevation of the thermal death-point of the organisms themselves has also been described by B. Fischer (1903, p. 289) who found that half-an-hour at 60° and even five minutes at 75° did not kill all the bacilli of a strain of paratyphoid B isolated by himself in an epidemic at Kiel. This observation I have not been able to confirm; young broth cultures of typhoid (two strains), paratyphoid A (two strains), paratyphoid B (four strains), Aertryck and Gaertner (two strains) all survived 10 minutes at 55° but were as uniformly killed by an exposure of similar length at 60°. Nor does the agglutinable substance of Gaertner bacilli appear to be more heat-resistant than that of typhoid. In my own experiments indeed it seems to be less thermostabile: heating broth cultures of *Gaertner L.I.P.M.* at 65° for 30 minutes greatly reduces or almost abolishes their agglutinability with both Gaertner and typhoid immune sera, while typhoid *Guy's* is far less affected. The results of one such experiment appears in the next table.

It would appear, then, that a good diagnosis may in most instances be made from the examination of the serum alone as to the limits and morphology of its agglutinins, their relation to heating and their combinations with bacillary bodies. Jürgens (1904) has shown that useful results in doubtful cases may be obtained by the direct estimation of the protective power of the serum in the peritoneal cavity of animals. I have not made any observations of the same

removed by filtration or centrifugalisation. This applies especially to human blood; it is not nearly so marked in rabbit blood.

kind, and Böhme (1905) shows that the reaction is not highly specific among members of this group of organisms.

Broth cultures 20 *hours: mixture of typhoid* A. E. Wright, *paratyphoid A* Brion and Kayser, *paratyphoid B* Schottmüller B, *and* Gaertner original A *immune rabbit sera.*

		Typhoid *Guy's*	*Brion and Kayser*	Paratyphoid B *Potts*	*Gaertner L.I.P.M.*
Before heating	1 : 50	+ + +	+ + +	+ + +	+ + +
	1 : 500	+ + +	+ + +	+ + +	+ + +
	1 : 1,000	+ + +	+ + +	+ + +	+ + +
	1 : 5,000	+ +	+ +	+	+ +
Heated at 60° for 30 mins.	1 : 50	+ + +	+ + +	+ + +	+ + +
	1 : 500	+ + +	+ + +	+ + +	+ + +
	1 : 1,000	+ + +	+ + +	+ + +	+ + +
	1 : 5,000	+ +	+ +	+	0
Heated at 70° for 30 mins.	1 : 50	+ + +	+ +	+	+ +
	1 : 500	+ + +	+ +	+	0
	1 : 1,000	+ +	+ + +	0	0
	1 : 5,000	+ +	+	0	0

VI. *Details of cases observed.*

Case I. "*Potts*" (28. iii. '05). Male 8 years. Clinically mild typhoid; onset sudden: duration of fever three weeks. During the first week the blood was examined twice; on both occasions it gave complete agglutination with *Schottmüller B*, but no reaction with typhoid or *Schottmüller A*, at 1 : 50 (higher dilutions not tried). In early convalescence organisms corresponding culturally with *Schottmüller B* were easily obtained by direct plating of hard scybalous stools, both from the surface and the interior of the faecal masses. A third sample of blood obtained a month after the fever had ceased gave the following agglutination limits:

Typhoid *Guy's*	100	*Gaertner L.I.P.M.*		<100
Schottmüller A	<100	Organisms isolated from stools	No. 8	10,000
Schottmüller B	20,000		No. 12	20,000
Aertryck	20,000		No. 29	10,000

Case II. "*Preston*" (14. vi. '05). Male $1\frac{10}{12}$ years. Clinically indefinite but cerebral symptoms predominated and patient was thought to have "meningitis or influenza with nervous symptoms." Onset gradual. Duration about $2\frac{1}{2}$ weeks; contracted varicella in fourth week. At the end of the second week serum completely

agglutinated *Schottmüller B* at 1 : 1,000 but was negative with typhoid and *Schottmüller A* at 1 : 50. From the stools nothing resembling paratyphoid could be obtained by plating direct or after preliminary incubation in dextrose-bile-salt peptone water: after incubation in dulcite medium a fair number of colonies were obtained corresponding culturally with *Schottmüller B*. A second sample of blood obtained in the fourth week agglutinated *Schottmüller B* and the organisms obtained from the stools up to 1 : 10,000.

Case III. "*Barkley*" (28. vii. '05). Male 13 years. Clinically mild typhoid; onset gradual, commencing while patient was in Dublin. The blood examinations gave:

	Typhoid *Guy's*	Typhoid *Lincoln*	*Schott. A*	*Brion and Kayser*	*Schott. B*	*Aertryck*	*Gaertner L.I.P.M.*
8th day of disease	100	—	50	—	>1,000	500	50
11th ,, ,,	200	200	50	500	>5,000	1,000	200

Absorption experiments with the two samples gave the same results; the details of the first sample are as follows; those of the second being given on p. 56.

	Agglutinations (after absorption) at 1 : 20 with				
Absorbed at 1 : 10 with	Typhoid *Guy's*	*Schott. A*	*Schott. B*	*Aertryck*	*Gaertner*
Typhoid *Guy's*	0	0	+ + +	+ + +	0
Schottmüller A	0	0	+ + +	+ + +	0
,, *B*	0	0	0	0	0
Aertryck	0	0	+ + +	0	0
B. coli communis	+	tr	+ + +	+ + +	+ +

From a specimen of faeces during the third week a single colony corresponding culturally to *Schottmüller B* was obtained after preliminary incubation in dulcite peptone water; many were obtained from the blood of guinea-pigs killed by the intraperitoneal inoculation of dulcite cultures of the stools. In a second sample of stools obtained in the fifth week no organisms resembling paratyphoid could be found.

It will be convenient to describe here together the agglutination reactions of the organisms isolated from these three cases with sera derived from rabbits inoculated with known cultures:

Serum	Typhoid *Guy's*	*Schott. A*	*Schott. B*	*Aertryck*	*Gaertner L.I.P.M.*	*Potts*	*Preston*	*Barkley*
Schott. B	200	50	5,000	200	200	2,000	5,000	5,000
Aertryck	200	0	100	5,000	0	50	100	50
Gaertner original A	1,000	50	0	0	5,000	0	0	0

These results show that the organisms obtained belong clearly to the paratyphoid B (*Schottmüller B*) rather than to the *Aertryck* (hog-cholera) or Gaertner (food-poisoning) group.

Their pathogenicity was typically of a high order. Guinea-pigs of about 250 gms. were inoculated with the following amounts of 20 hours' broth cultures incubated at 37° C.:

	Potts		*Preston*		*Barkley*	
	Subcut.	Intraperit.	Subcut.	Intraperit.	Subcut.	Intraperit.
1 c.c.	dead < 18 hrs.	—	dead 40 hrs.	—	—	—
0·5 c.c.	—	—	—	—	lived	dead < 18 hrs.
0·1 c.c.	dead 40 hrs.	dead < 18 hrs.	dead 12 days *	dead < 18 hrs.	dead 7 days	dead < 18 hrs.
0·01 c.c.	dead 20 hrs.	dead 20 hrs.	dead 40 hrs.	dead < 18 hrs.	dead 50 hrs.	lived
0·001 c.c.	—	dead 28 hrs.	—	—	lived	dead 42 hrs.

* Abscess at site of inoculation; paratyphoid B recovered from heart blood.

Feeding experiments were negative. Two guinea-pigs were fed twice with green food soaked in broth cultures of *Potts*, and two rats and two mice lived for some days on the organs of guinea-pigs killed by inoculation with the same organism. Though the organisms were found in abundance in the faeces of the guinea-pigs a week later, no symptoms of illness were observed, and all the animals were alive and well after two months.

Case IV. *Valérie* (1. viii. '05). Female 45 years. Clinically somewhat atypical typhoid; duration three weeks. The blood was examined in the first, second and third weeks:

	Agglutination limits with								
	Typhoid *Guy's*	Typhoid *Lincoln*	*Schott. A*	*Brion and Kayser*	*Schott. B*	*Aertryck*	*Gaertner L.I.P.M.*	*Valérie* 21 (see below)	*Valérie* 25 (see below)
1. viii. '05	1,000	—	0	1,000	5,000	—	1,000	—	—
8. viii. '05	500	1,000	0	200	5,000	200	100	—	—
15. viii. '05	1,000	2,000	0	1,000	> 20,000	1,000	200	1,000	0

These results indicate fairly clearly a paratyphoid B infection. The absorption experiments gave however anomalous results; with the second sample the whole of the agglutinins could not be exhausted with typhoid *Guy's*, *Brion and Kayser*, *Schottmüller B* or *Aertryck*. The details for the third sample are given, the chief combinations having been confirmed by absorption in other dilutions:

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Absorbed at 1:50 with	Agglutinations (after absorption) at 1:100 with							
	Typhoid *Guy's*	Typhoid *Lincoln*	*Brion and Kayser*	*Schott. B*	Parat. B *Potts*	*Aertryck*	*Gaertner L.I.P.M.*	*Valérie*
Typhoid *Guy's*	0	0	+ +	+ + +	—	+ + +	0	—
Typhoid *Lincoln*	0	0	0	+ + +	—	+ + +	0	—
Brion and Kayser	0	+ +	0	+ + +	—	+ + +	0	+ + +
Schottmüller B	0	0	+ + +	0	—	0	0	+ + +
Paratyphoid B, *Potts*	0	0	+ +	0	0	0	0	+ +
Aertryck	0	+ + +	+ +	+ + +	—	0	0	—
Gaertner L.I.P.M.	0	+ +	+ + +	+ + +	—	+ + +	0	—
B. coli communis	+ + +	+ + +	+ + +	+ + +	—	+ + +	0	—
Valérie 21	0	+ +	0	+ + +	+ + +	+ + +	0	0
Schottmüller B + *Brion and Kayser*	0	0	0	0	0	0	0	+ + +

Disregarding the results as far as they concern *Valérie* 21, it would appear from this table that the case was one of mixed infection with paratyphoid A and B, or with paratyphoid B and some unknown organism giving rise to secondary agglutinins for *Brion and Kayser* (see above p. 59).

The stools were examined on two occasions for paratyphoid organisms but none could be found by any method. Two varieties of dulcite-fermenting, non-lactose-fermenting "*coli*-like" organisms were however present in some numbers and require mention.

(*a*) "*Valérie* 25": actively motile; no liquefaction of gelatin; fair quantity of indol; milk strongly acid, but unclotted after 19 days;

acid and gas produced in	no apparent change in
dextrose	*lactose*
laevulose	*cane-sugar*
maltose	raffinose
galactose	*sorbite*
arabinose	erythrite
dextrin	amygdalin
mannite	inulin
dulcite	
salicin	

At first sight this organism is very similar to *Schottmüller A* or *Brion and Kayser*. It differs however culturally from these organisms in its rapid and extensive fermentation of salicin and in its failure to attack sorbite. With the serum of rabbits inoculated with *Schottmüller A* or *Brion and Kayser* it shows no agglutination even in low dilutions, and the cultures from the stools of the present case entirely failed to

react with the patient's, or indeed any other, serum at 1 : 20. The same organism was isolated in large numbers from the urine and faeces of the next case (*Shepherd* 1). The pathogenicity was not high; a 200 grm. guinea-pig survived the intraperitoneal injection of 1 c.c. of a 24 hours' broth culture of *Valérie* 25; of *Shepherd* 1 0·5 c.c. killed in 18 hours, but 0·1 c.c. was not fatal. This bacillus appears indeed to be very close to the "B. paratyphoid A, unknown type," obtained by Morgan (1905, p. 1260) from the faeces and intestines of animals and from milk, and is probably a not uncommon intestinal organism. Dr Morgan has been good enough to furnish me with a culture of his bacillus; I find that it ferments salicin readily and fails to attack sorbite in 48 hours; acid and gas is however produced in 7 days. Its pathological relations are unknown; possibly it has none. It is however of interest as bearing a somewhat deceitful resemblance to paratyphoid A.

(β) "*Valérie* 21": actively motile; no liquefaction of gelatine; fair quantity of indol; milk rapidly acidified and clotted;

acid and gas formed in	no apparent action on
dextrose	*lactose*
laevulose	erythrite
maltose	*salicin*
galactose	amygdalin
cane-sugar	inulin
arabinose	
raffinose	
dextrin	
dulcite	
mannite	
sorbite	

A 200 grm. guinea-pig died overnight after receiving 0·1 c.c. of a 24 hours' broth culture intraperitoneally. This organism has several radical cultural distinctions from the paratyphoid group, and only finds mention here on account of its agglutinative reactions. With the patient's serum (15. viii. '05) it gave a complete reaction up to 1 : 1000; after admixture with excess of this bacillus, the serum lost not only this reaction but also failed to agglutinate *Brion and Kayser*, the agglutinin for which could not be removed by the organism (*Schottmüller B*) which was predominant in the agglutinations. On the other hand, the reaction

with *Valérie* 21 was not removed by treatment with *Schottmüller B* and *Brion and Kayser* which together removed all the other agglutinins[1]. These results suggest that the condition in the present case was possibly one of double infection with paratyphoid B and *Valérie* 21, the latter being the source of the *Brion and Kayser* agglutination, and the former having secondary agglutinins for *Aertryck* and typhoid *Lincoln*. The phenomena however do not necessarily imply that *Valérie* 21 had any aetiological connection with the present case. It seems to the writer that they are otherwise explicable on the grounds (1) that the organism in question is one of very high agglutinability; it has in fact been found that it agglutinates at 1 : 200 to 1 : 500 with *Schottmüller B*, *Brion and Kayser*, *Aertryck* and typhoid rabbit sera (agglutinating their homologous bacilli at 1 : 1,000 to 1 : 10,000), showing at the same time no trace of agglutination in normal saline: (2) that such an organism, differing widely in its cultural reactions from the group to which the sera belong, may not altogether conform to Castellani's rule in absorption experiments. As an example its behaviour in the serum of a rabbit inoculated with *Schottmüller B* may be quoted:

	Agglutination (after absorption) at 1:20 with							
Absorbed at 1:10 with	Typhoid *Guy's*	Typhoid *Lincoln*	*Brion and Kayser*	*Schott. B*	Parat. B *Potts*	*Aertryck*	*Gaertner L.I.P.M.*	*Valérie* 21
Typhoid *Guy's*	0	0	+ + +	+ + +	+ + +	+ +	0	+ +
Brion & Kayser	+	0	0	+ + +	+ + +	+ + +	0	+ +
Schott. B	0	0	0	0	0	0	0	+ +
Valérie 21	+ +	+	+ + +	+ + +	+ + +	+ + +	0	0
Original titre	1,000	500	5,000	10,000	10,000	200	50	200

On the other hand the failure of this organism to conform to the rules of absorption does not always hold good. With a *Brion and Kayser* rabbit serum the following results were obtained:

	Agglutination at 1:50 with		
Absorbed at 1:25 with	Typhoid *Guy's*	*Brion and Kayser*	*Valérie* 21
Typhoid *Guy's*	0	+ + +	+ + +
Brion and Kayser	0	0	0
B. coli	0	+ + +	+ +
Original titre	500	1,000	100

The exact nature of this case remains uncertain. The high agglutination value of the third sample of serum with *Schottmüller B* (1 : 20,000)

[1] The mode of expression is convenient; it must not be taken as implying that these various reactions depend on the presence in the serum of a variety of substances rather than of a substance with a variety of properties.

renders it difficult to escape from the conclusion that there was an infection with paratyphoid B: the similarity of the behaviour of *Valérie* 21 in the patient's serum and in the artificial *Schottmüller B* serum may also be not without significance. Whether a second infection was superadded, and if so with what organism, remain undecided.

Case V. "*Shepherd*" (6. ix. '05). Male, 4 years; clinically atypical typhoid. Two samples of blood gave the following agglutination limits:

	Typhoid *Guy's*	*Brion and Kayser*	*Schott. B*	*Aertryck*	*Gaertner L.I.P.M.*
6. ix. '05	50	0	>500	50	0
14. ix. '05	0	0	10,000	50	0

Repeated attempts were made to isolate paratyphoid bacilli from the urine and faeces by plating and by animal inoculations, but without success. The organism already described under Case IV. (*Valérie* 25) as resembling paratyphoid A was present in large numbers in urine and faeces: it gave no reaction with the patient's serum. The high, and almost pure, agglutination with *Schottmüller B* renders the diagnosis nearly certain.

Summary.

1. Three fully-established, and two probable, cases of infection with *B. paratyphosus B* producing a typhoid-like illness have been found in a series of 176 cases of typhoid.

2. Preliminary culture in a fluid medium containing dulcite is of great assistance in isolating paratyphoid organisms from mixtures such as faeces.

3. The diagnosis of paratyphoid fever can generally be made from the agglutinative reactions of the serum.

4. Absorption tests are necessary for the positive or negative diagnosis of mixed infections.

5. The structure of the agglutinated masses of paratyphoid and Gaertner bacilli varies with homologous and heterologous sera, and is of diagnostic import.

6. Gaertner and paratyphoid agglutinins are more thermostabile than typhoid agglutinin by 5°—10° C.

7. The agglutinations of paratyphoid, hog-cholera and Gaertner bacilli with a series of typhoid sera are described, and the circumstances which influence the presence and amount of these secondary agglutinins discussed.

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Literature[1].

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[1] No attempt has been made to give a complete bibliography; many references will be found in Buxton (1902), Pratt (1903), Trautmann (1903) and Kayser (1904 *b*).

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