SECOND REPORT ON BACTERIOLOGICAL ASPECTS OF THE MENINGOCOCCUS CARRIER PROBLEM[1].

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INTRODUCTION.

In my first report[1] I showed that in the throats of persons not known to have been in contact with cases of cerebro-spinal fever organisms were frequently found which were indistinguishable from meningococci isolated from the cerebro-spinal fluid of persons suffering from that disease.

During 1916 the investigators in the Board's Laboratory have continued to make further bacteriological inquiry into the characteristics of such naso-pharyngeal organisms, and into their relationship to strains obtained from the cerebro-spinal fluid in cases of meningococcal meningitis. In addition to Drs F. Griffith and W. M. Scott, who have been occupied with this research throughout the year, Captain C. W. Ponder, R.A.M.C., has taken part in the investigation since June, 1916, when he was loaned to the Board by the War Office for this purpose.

Before discussing scientific details it will be useful to set out the main objects of the present work in general terms.

With the view of ascertaining whether further investigation would corroborate the previous year's findings, more naso-pharyngeal swabs from non-contacts have been examined, and this inquiry has been extended into localities distant from those previously investigated. The results have been confirmatory of the previous work, in that many additional non-contact strains have been found which are indistinguishable from meningococci of cerebro-spinal origin.

Whilst these facts point to a wide distribution of the meningococcus amongst the population of the areas investigated, it is necessary, before drawing general conclusions, to consider whether the tests applied for the identification of this organism have been sufficient, and whether some test might not be found which would serve to differentiate meningococci of cerebro-spinal origin from the majority, at least, of those which occur in the throats of non-contacts. With this object Drs Griffith and Scott and Captain Ponder have paid special attention to serological reactions, as possibly affording a means of differentiation.

The problem would be simple if it were possible to adopt one meningococcal serum as the standard and to lay down the law that naso-pharyngeal strains which are agglutinated by this serum are meningococci, and that strains which are not agglutinated by it are not meningococci. But any such simple solution is quite out of the question. It is clear from the last reports by the Board's investigators that undoubted

[1] *Journ. of Hygiene*, xv. 405.

meningococci of cerebro-spinal origin differ among themselves in their serological reactions, and that no one serum is available which will agglutinate them all. This fact has been amply confirmed by other bacteriologists.

Can the difficulty be met by using a larger number of standard meningococcal sera, on the hypothesis that, if each variety of true meningococcus be represented serologically, an organism which agglutinated with at least one of the sera would be a meningococcus, whilst failure to agglutinate with at least one serum would exclude an organism from this class?

This hypothesis raises important issues which must first be clearly defined, and then be examined in the light of laboratory data.

(1) How many standard sera would be required? If two or three would suffice, it would not be impracticable to test each unknown strain against each serum; but if a large, and perhaps indefinitely large, number of sera would be needed, the method would be impracticable for routine diagnosis.

(2) Would it be possible to establish identity of standards? One laboratory might adopt a certain set of sera as being the most useful for differentiation, and another laboratory, with equal right of scientific authority, might adopt a different set, which might give different results. Then the decision as to what was or was not a meningococcus would be no more than the expression of a personal opinion, and would vary according to the views of the particular investigator.

(3) Would the results of simple agglutination tests necessarily be diagnostic? One knows that for certain organisms—e.g. the typhoid bacillus and the cholera vibrio—the ordinary agglutination test is extremely useful, and, indeed, invaluable; and though the bacteriologist has to keep on the alert for possible fallacies, the need for caution does not detract from the fact that, with these organisms, the agglutination test is of very great practical utility. But agglutination with a coccus is much more irregular, and so many precautions have to be taken to avoid error that the question arises, certainly in the case of the meningococcus, whether the result of a simple agglutination test can be accepted as a final criterion for routine diagnosis.

(4) Is there any way of improving the agglutination test so as to overcome the difficulties met with in the meningococcus? The difficulties are twofold, and are similar to the difficulties encountered in serological tests with other organisms. Sometimes (a) a standard meningococcus serum may agglutinate organisms which are not meningococci—e.g. it

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may agglutinate gonococci; and sometimes (*b*) an undoubted meningo-coccus may fail to agglutinate with any standard serum, just as a pneumococcus may fail to agglutinate with any standard pneumococcal serum, although it may be known to have produced lobar pneumonia. A suggested way out of the difficulty (*a*) is based on the principle that if a serum prepared from an organism A agglutinates not only A but also a different organism B, these two different capacities of the serum can be separated out by treating the serum with a culture of B and then removing the deposit; the clear liquid remaining will be found to have lost its power of agglutinating B but to have retained its power of agglutinating A. If the serum had been treated with a culture of A, instead of B, and then retested, its power of agglutinating A would have disappeared. This indicates a method of ascertaining whether an unknown organism which is agglutinated by serum A (a meningococcal serum) is identical with A (a meningococcus) or is really some different organism (B). It also suggests (*b*) a method of ascertaining whether a culture of an unknown organism which is not agglutinated by serum A is of the same type as culture A; if it is, the serum, after treatment with the culture in question, will have lost its power of agglutinating culture A; but if it is not, the capacity of the serum for agglutinating culture A will not be affected. These considerations naturally raise the question whether the proposed method of differentiation by "the absorption of agglutinin test" is reliable for the identification or differentiation of meningococci.

(5) The question last raised involves general principles of bacterio-logical classification, and the answer to it must involve consideration of these. The definite issue raised is whether the principle of differentia-tion by capacity for absorption of agglutinin is valid for the classification of organisms which, in other bacteriological respects, are essentially indistinguishable from each other. The practical importance of this problem may be illustrated by a well-known example. In certain in-testinal disorders and in suspected food-poisoning there are two organisms, amongst others, which it is important to identify, viz. *B. paratyphosus* (B) and a widely-distributed organism known as *B. suipestifer*. These two are closely allied in their bacteriological reactions, and a good deal of research has been devoted to the question of their inter-relationship, both in England and in Germany. Opinion is still divided between two opposite schools, the one holding that the two organisms are clearly distinguishable by the absorption method, whilst the other maintains that both belong to the same group, and that they cannot be differentiated

either by absorption or by any other bacteriological means. This illustration is particularly apposite to the question whether meningococci can be divided into (a) a pathogenic class and (b) a common but relatively harmless saprophytic class, because in the meningococcal problem the questions of scientific methods of differentiation and classification are identical with those raised in the controversy regarding *paratyphosus* and *suipestifer*.

(6) So far, I have only referred to the diagnosis of non-contact strains by means of sera prepared from cerebro-spinal meningococci. What further evidence of inter-relationship or differences would be obtained if sera were prepared from naso-pharyngeal strains and were fully investigated? Such information is obviously required, though the preparation of sera from unknown strains would not be practicable as a part of routine diagnosis.

(7) All these questions mean that serological diagnosis may be inaccurate unless it is based upon a correct appreciation of the limitations of the method employed.

It is therefore necessary to examine current assumptions about the principles of immunity, in so far as they affect the interpretation of certain serological reactions, and to enquire into the validity of their application to the differentiation of species.

(8) After due consideration of the above questions the final issue as to the diagnostic value of serological tests may be brought to a focus. The crucial question will be—a naso-pharyngeal strain is tested serologically and is found to give reactions which do not identify it as a meningococcus though it is identical with meningococci morphologically, culturally, and in fermentation tests. Is such an organism to be regarded as possibly capable of producing cerebro-spinal fever? And if the answer is in the negative, the nature of the required serological identity must be defined.

THE DISTRIBUTION OF THE MENINGOCOCCUS AMONGST THE GENERAL POPULATION.

THE USE OF THE TERM "NON-CONTACT."

With reference to the work of the Board's investigators on the carrier problem it will be useful to clear up certain ambiguities attaching to the significance of the convenient terms "contact" and "non-contact."

There is, I believe, general agreement on the following matters:—

(1) Cerebro-spinal fever develops in persons who, prior to the onset

of the disease, have "carried" the meningococcus in their naso-pharynges.

(2) The number of persons who develop the disease is very small in proportion to the number of carriers.

(3) The meningococcus is disseminated amongst the population by contact with carriers.

(4) When a person develops cerebro-spinal fever, some of the persons who have been in intimate contact with him will also, in all probability, be found to be carriers. Such carriers may be termed, collectively, Group I.

(5) It cannot be assumed that each member of Group I became a carrier owing to contact with the person who developed the disease; it is quite possible that the patient derived his infection from one of these healthy carriers.

(6) Persons not in contact with the patient may have been in contact with one or other of the persons A, B, C, etc., who constitute Group I, and may have become carriers in consequence, thus forming Groups A, B, C, etc.

(7) Similarly each individual in Groups A, B, C, etc. may be the focus of another group; and so the process may go on indefinitely.

(8) Carriers may retain the meningococcus in their throats for a long time, though not, as a rule, for more than two or three weeks.

It is thus evident that a case of cerebro-spinal fever can usually be regarded as associated, directly and indirectly, with an indefinitely large number of carriers, of whom (a) some are known to have been in contact with the patient; (b) a larger number can be found on enquiry to have been associated, directly or intermediately, with (a); and (c) a still larger number are intermediately connected with (a), but the connecting links cannot be traced. Then the rest of the population would comprise (d) all the persons, whether carriers or not, who have no connecting links, however remote or obscure, with (a).

To avoid ambiguity, therefore, the distinction between "contacts" and "non-contacts" should be expanded into a distinction between (a) direct contacts (known), (b) indirect contacts (known), (c) unknown contacts (direct or indirect) and (d) persons who have not been contacts either directly or indirectly.

Turning now to the practical problem, what is wanted is to ascertain the distribution of the meningococcus in the general population, and for that purpose the population has to be "sampled." It is already known that a good many people who have been associated, directly or indirectly,

with cerebro-spinal fever are carriers, but as it cannot be taken for granted that these persons are a representative sample of the general population, the "samples" must be taken from persons unconnected, so far as is known, with the disease. If it turns out that amongst these people, conveniently termed "non-contacts," carriers are few in number, the occurrence of such may possibly be explained on the hypothesis that they really belong to class (c), the "unknown contacts." But if, amongst such "non-contacts," carriers are found in considerable percentage, the hypothesis that they are really indirect but undiscovered cases of contact with the disease is of no assistance; it can neither be confirmed nor refuted, nor does it affect the main fact of practical importance that carriers are freely distributed amongst the normal population.

INTERPRETATION OF NEGATIVE RESULTS.

The incidence of meningococci in the naso-pharynx is probably very irregular, varying in different localities and in different seasons, and therefore it is not surprising that there are marked differences in the percentages of "positives" found by different observers. And, apart from irregularities in the incidence of the cocci, it must also be generally recognised that the conditions under which the swabs are taken and investigated influence the results very materially. In taking the swab, avoidance of contamination with the common bacteria of the mouth is particularly important, because, as recently shown by Colebrook (*Lancet*, Nov. 20th, 1915), and Gordon (*British Med. Journ.*, June 17th, 1916), certain organisms commonly present in the mouth inhibit the growth of the meningococcus. In plating out the swabs, a liberal supply of medium is necessary in order to allow the development of discrete colonies, since the meningococcus, unlike hardier organisms such as the diphtheria bacillus, will either not grow at all or will not form recognisable colonies if it is surrounded and overrun by a confluent growth of other organisms. In preparing the medium for primary culture, enrichment with some adjuvant such as serum or ascitic fluid is necessary to promote the growth of naso-pharyngeal meningococci. The necessity for this has always been recognised by most bacteriologists, and recent investigations have served to emphasise its importance[1]. And, above all, plenty of time and care are necessary at every stage in the process,

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[1] It must not be assumed that a medium which gives a good primary culture of cerebro-spinal meningococci is necessarily good for naso-pharyngeal meningococci; the cerebro-spinal fluid transferred to the plate along with the former organisms is itself an adjuvant to growth.

from the taking of the swab to the searching of the plate and the examination of suspicious colonies.

The above considerations must be taken into account when interpreting negative results, as these would lose their significance if they were not obtained under conditions specially favourable to the discovery of any meningococci possibly present. And, it may be necessary to point out, cases in which the cultures are overgrown should be separately recorded as such, and should be eliminated from the figures on which the percentage of positives is based.

Non-Contact Carriers at St Bartholomew's Hospital.

In continuation of the work recorded in my previous report (pp. 442–4), Mr C. E. West, F.R.C.S., Aural Surgeon to St Bartholomew's Hospital, took naso-pharyngeal swabs from two further series of out-patients, and sent the material to the Board's Laboratory for bacteriological examination. As before, the patients were taken as general examples of hospital out-patients or convalescents, and had not, so far as could be ascertained, been in contact with any cases of cerebro-spinal fever. The same technique of investigation was observed as in the previous year's work. The results were as follows:—

I.—*Cultural Tests of* 100 *Naso-pharyngeal Swabs taken Jan. 10th—24th*, 1916.

Age period	Males		Females		Totals (Male and Female)	
	Positive	Negative	Positive	Negative	Positive	Negative
0–5 years	0	6	0	5	0	11
5–10 ,,	1	10	0	8	1	18
10–20 ,,	0	14	1	17	1	31
20–40 ,,	2	10	1	10	3	20
Over 40 ,,	1	6	3	5	4	11
	4	46	5	45	9	91

In two of the nine positives, the original plates yielded pure cultures of meningococci; in four, the colonies of meningococci were numerous or moderately numerous; and in the remaining three the colonies were scanty.

II.—*Cultural Tests of* 100 *Naso-pharyngeal Swabs taken April 6th—June 5th*, 1916.

Age period	Males		Females		Totals (Male and Female)	
	Positive	Negative	Positive	Negative	Positive	Negative
0–5 years	0	2	0	1	0	3
5–10 „	0	5	2	3	2	8
10–20 „	8	12	3	10	11	22
20–40 „	8	7	5	8	13	15
Over 40 „	6	8	2	10	8	18
	22	34	12	32	34	66

In one of the thirty-four positives, the original plates yielded a pure culture of meningococci; in twenty-five, the colonies of meningococci were numerous or moderately numerous; and in the remaining eight the colonies were scanty.

Two swabs, not included among the above, are interesting. One was taken on May 4th from a female patient, aged 53, suffering from Eustachian catarrh, and yielded numerous colonies of meningococci; on January 13th a swab from the same person had given a practically pure culture of meningococci. On May 4th a swab taken from a female patient, aged 52, suffering from pharyngitis, gave moderately numerous colonies of meningococci; a previous swab, taken on January 20th, had been found positive, with numerous colonies of meningococci.

On setting out the whole of the St Bartholomew's Hospital results in successive batches of 100, the data are:—

Period	Number of Positives
March 29th—April 19th, 1915	20
April 19th—May 6th, 1915	7
May 6th—June 7th, 1915	6
June 7th—June 24th, 1915	7
*June 24th—July 22nd, 1915 . . .	†11
January 10th—January 24th, 1916 . . .	9
April 6th—June 5th, 1916	34

* *Only* 80 *cases examined.* † *Percentage.*

CARRIERS IN KENT, CAMBRIDGE, AND NORWICH.

Dr Scott has examined both contacts and non-contacts in East Kent, and has reported his results on pp. 240–5; and Captain Ponder has investigated the non-contact populations of Cambridge and Norwich (pp. 247–280).

It will be seen from Dr Scott's report that a high percentage of persons carrying undoubted meningococci was found amongst non-contacts as well as amongst contacts; and Captain Ponder has shown that similar organisms were found in high percentage amongst non-contacts, including healthy workpeople, at Cambridge and Norwich. These results are in conformity with the figures obtained for London.

VALUE OF SIMPLE AGGLUTINATION TESTS AS AN AID TO DIAGNOSIS.

Is there a Pseudo-Meningococcus?

As I showed in my last report, many investigators have used the prefix "pseudo" without adequate justification. Apparently they took it for granted that genuine meningococci would not be found in the throats of non-contacts, and some of them failed to appreciate the difficulties of serological diagnosis which arise from the fact that strains of undoubted meningococci are not necessarily agglutinated by the particular serum employed. Hence a non-contact strain, though indistinguishable culturally from the meningococcus, was branded with some such prefix as "pseudo" for the insufficient reason that it did not tally with certain standard strains in serological reactions. But since then it has been recognised that different strains behave differently towards different sera and exhibit a tendency to serological grouping. This is an important advance towards accurate identification and classification, and I note that, in view of this fact, the Medical Research Committee's Report[1] expresses the hope (p. 19) "that the terms 'para-' and 'pseudo-meningococcus' will in time be dropped."

But, whilst condemning arbitrary usage of the designation "pseudo," the above considerations are not enough to dismiss the important practical question:—Does the naso-pharynx harbour organisms which, though "meningococcus-like" and perhaps botanically related to meningococci, are incapable of producing meningitis?

In raising this question the position of organisms resembling the cholera vibrio may be considered as analogous. The search for the meningococcus in the human naso-pharynx may be compared to the search for the organism of cholera in an Indian water-tank, wherein there are frequently to be found vibrios which are "cholera-like" but are not true cholera. Greig, for example, has investigated a large

[1] *Report of the Special Advisory Committee upon Bacteriological Studies of Cerebro-Spinal Fever during the Epidemic of 1915.*

number of such cholera-like vibrios and has found[1] that they resembled true cholera culturally, but were not agglutinated by a high titre cholera serum, and did not produce agglutinins for the standard cholera vibrio. The last test he regards as important, because, as he has explained in a previous article[2], the true cholera vibrio may lose its agglutinability but does not lose its agglutinogenic capacity even if exposed to the action of water for a long period.

Reverting to the meningococcus, the parallel question will be:—

Are the organisms commonly found in the non-contact naso-pharynx distinguishable from cerebro-spinal meningococci in that they do not agglutinate well with any sera prepared by the latter and that they are incapable of producing sera which will agglutinate the latter?

On reference to the work of the Board's investigators it is clear that the great majority of non-contact meningococci do not answer to this description.

Dr Griffith has compared the agglutinability of 66 cerebro-spinal strains and 86 non-contact naso-pharyngeal strains with six sera prepared with spinal strains and has found (p. 132) that 94 per cent. of the former and 72 per cent. of the latter were agglutinated up to 400 or over with one or more of these sera. On preparing monovalent sera with six of his naso-pharyngeal strains, he found (p. 137) that these latter sera showed:—"(a) good agglutination with cerebro-spinal meningococci, though usually short of full titre; (b) more uniform influence on Group I strains; (c) agglutination of some of the Group II strains to half full titre." Supplementing these data with observations on a batch of more recently isolated cerebro-spinal strains, he has found (p. 190) that 19 out of 23 of these were agglutinated up to 400 or over by the serum prepared from his naso-pharyngeal strain NP 44 (titre 1 : 800).

Dr Scott examined 71 naso-pharyngeal strains. Of these (p. 230), 44 agglutinated with his Group II sera (30 up to full titre, 9 to 1,000; and 5 to 500; one of the last 5 also went up to 500 with a Group I serum). Of the remaining 27, 14 agglutinated with Group I sera (2 up to 1,500, 2 up to 1,000, and 10 up to 500). As regards agglutinogenic capacity, 8 of these 14 naso-pharyngeal strains produced sera agglutinating certain cerebro-spinal members of Group I. Agglutinogenic capacity of naso-pharyngeal strains resembling cerebro-spinal members of Group II was not investigated.

[1] The Serological Investigation and Classification of Cholera-like Vibrios isolated from water in Calcutta. *Indian Journ. of Med. Research*, April, 1916.

[2] *Ibid.*, Jan., 1916.

Captain Ponder, who did not find that his cerebro-spinal strains were clearly separable into two main groups, tested the agglutinability of 94 non-contact naso-pharyngeal strains with sera prepared from cerebro-spinal strains, and found that 74 per cent. of these non-contact strains "gave evidence of relationship to the meningococcus in virtue of their agglutination reactions" (p. 280). He tested the agglutinogenic capacities of two of his naso-pharyngeal strains, Nos. 108 and 235, towards 16 spinal strains; 13 of these were agglutinated to 400 or over with the serum prepared from the former (titre about 1 : 400), and 9 of these 13 were also agglutinated to 400 or over by NP 235 serum (titre about 1 : 800).

Comparison between the Non-Contact Meningococcus and the Pneumococcus "Carried" by Normal Individuals.

Whilst recognising that there is no evidence of a valid analogy between water vibrios which are merely "cholera-like" and prevalent strains of non-contact meningococci, it might be urged that a better analogy is provided by recent serological work on the pneumococcus, the outcome of which is to suggest that the pneumococci commonly met with in the mouths of normal individuals, though genuine pneumococci and not merely "pneumococcus-like," are distinguishable from the majority of pneumococci which have been responsible for lobar pneumonia or other acute infection. As this is an important suggestion, which was raised a year ago in the Medical Research Committee's Report, it calls for consideration in the light of laboratory data.

In 1910, Neufeld and Haendel[1] called attention to the occurrence of pneumococci which did not agglutinate with standard sera, and expressed the opinion that extensive enquiry ought to be made into the prevalence of special types of pneumococci and into the occurrence and distribution of atypical strains.

Recognising the importance of the problem raised by Neufeld and his associates, Dochez and Gillespie (1913)[2] attempted to form a biological classification of pneumococci by means of immunity reactions. They investigated the pneumococci derived from 74 cases of typical lobar pneumonia and grouped them as follows:—

[1] *Arb. aus d. Kaiserl. Gesundheitsamte*, xxxiv. 293.
[2] *Journ. American Med. Assoc.*, lxi. 727.

Group	No. of Cases	Percentages
I.	35	47
II.	13	18
III. (*mucosus*)	10	13
IV. (heterogeneous)	16	22

As the Group III organism, the *Pneumococcus mucosus*, is distinguishable culturally from the pneumococci in the other groups, it does not concern the meningococcus problem. Omitting this group, there are left 64 cases, of which 35 (55 per cent.) fall into Group I, 13 (20 per cent.) into Group II, and 16 (25 per cent.) into Group IV. Groups I, II and IV were indistinguishable morphologically and culturally, but serological tests gave the following results. A Group I serum protected white mice against all Group I strains, but not against any strains of II or IV, and this serum also agglutinated all Group I strains, but no strains of II or IV. Similarly, *mutatis mutandis*, with Group II. Group IV is not a group in the same sense as the other two. It is the residue, and comprises organisms which all differ serologically from each other as well as from those in I and II. "This group comprises a number of distinct varieties of pneumococcus which cannot be related to one another by immunologic reactions. Culturally they are true pneumococci, and manifest all the common characters of pneumococcus." The authors go on to suggest that the other groups comprise the "fixed races," which "are more highly parasitic and are never very far removed from a condition of pure parasitism, whereas the heterogeneous strains may be representatives of the types of pneumococcus found in the normal mouth, and consequently more likely to have undergone environmental changes."

In continuation of the above work, Dochez and Avery (1914 and 1915)[1] examined the pneumococci from 71 additional cases of lobar pneumonia and classed them as follows:—

Group	No. of cases	Percentages
I.	21	30
II.	28	39
III. (*mucosus*)	6	8
IV. (heterogeneous)	16	23

[1] *Journ. Exper. Med.*, xxi. 114 and xxii. 105.

Omitting Group III and combining the remaining data with those previously recorded, out of 129 cases of lobar pneumonia, there were 56 (43 per cent.) in Group I, 41 (32 per cent.) in Group II, and 32 (25 per cent.) in Group IV. The authors noted that the case mortality was lower in Group IV than in any of the other groups.

They also studied the pneumococci in convalescents from pneumonia, in healthy contacts, and in the sputum of non-contacts. In convalescents they found that generally the "fixed types," I–III, disappeared, and were replaced by IV, though sometimes a convalescent was a carrier of a "fixed type" for a long time. In healthy contacts the "fixed types" were often found. About 60 per cent. of the mouths of normal persons (not contacts) yielded pneumococci, but these organisms all belonged to Group IV.

With reference to the pneumococci in each of their four groups the authors say that "up to the present time we have observed no tendency of these organisms to lose their specific characters, nor have we observed a change of one type into another."

Cole (1915)[1] gives some further information as to the relative virulence of the four groups in cases of pneumonia, viz.:

Group	Cases	Deaths	Mortality Percentage
I.	28	7	25
II.	25	9	36
III.	17	8	47
IV.	33	2	6
	103	26	25

He also quotes the corresponding mortality statistics from the Pennsylvania Hospital, which show 29 per cent. for Group I, 27 for Group II, 67 for Group III, and 11 for Group IV.

Stillman (1916)[2] summarises for the four years 1912–13 to 1915–16 the types of pneumococci isolated from cases of lobar pneumonia admitted to the hospital of the Rockefeller Institute:—

Type	Number of cases	Percentage
I.	105	33·54
II.	99	31·62
III.	35	11·18
IV.	74	23·64

[1] *New York Med. Journ.*, Jan. 2, 1915, p. 1
[2] *Journ. Exper. Med.*, xxiv. 651.

The above data raise a clear issue. Do non-contact meningococci resemble the American Group IV pneumococci?

As regards pathogenicity, the Group IV pneumococcus is said to be responsible for about 25 per cent. of the cases of lobar pneumonia, though these cases have a relatively low death-rate. If the non-contact meningococcus bears a similar relationship to meningitis, it is obviously very far from being a harmless saprophyte.

Serologically, Group IV pneumococci differ markedly from each other and show no relationship to Groups I or II. The data quoted above as to the serological reactions of non-contact meningococci and their relations to meningococci of cerebro-spinal origin show that it is impossible to make a serological subdivision of meningococci which would place the non-contact strains in an independent group, resembling the Group IV pneumococci as regards individual differences in agglutinability and agglutinogenic capacity, and differing from the other groups of pneumococci which are said to be found only in pneumococcal infections or in contacts therewith.

It would, however, be unsafe to draw the conclusion that there is no real parallel between the non-contact carrier of the pneumococcus and the non-contact meningococcus carrier. On the contrary, there appears to be an interesting, and probably a very important, parallelism between the two conditions; and the inference I would prefer to draw from the literature I have quoted is that the American theory needs independent re-investigation in this country and cannot, at present, be regarded as permanently established[1]. Perhaps the American investigators are already beginning to discover this.

In the recent article by Stillman, to which I have referred above, there is the significant statement that, though Types I and II are not found in the normal mouth except in the case of contacts, recent studies have shown that Type III is "fairly common in the mouth flora of healthy individuals and infections with organisms of this type may be autogenic in nature." Type III, he goes on to say, was found in 44 out of 398 normal persons (23·4 per cent.) whilst Type IV was found in 58·5 per cent. There was no serological difference between non-contact Type III and pneumonic Type III; and it was found that the former

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[1] F. S. Lister has found that in the pneumonia of South African miners there are at least four groups of pneumococci in addition to those recognised by the Americans. (The South African Institute for Medical Research. No. VIII. *An Experimental Study of Prophylactic Inoculation against Pneumococcal Infection in the Rabbit and in Man.* Published by the Institute, Oct. 1st, 1916.)

might persist in the normal mouth for a long time. Here one may remark that the wide distribution of Type III (*Pneumococcus* or *Streptococcus mucosus*) in normal individuals is not a new discovery. It is mentioned, for example, by Lingelsheim (1912)[1] as a well-known fact that this organism is a not uncommon inhabitant of the upper respiratory tract.

Since Type III, the most virulent of the so-called "epidemic" types, turns out to be an organism which quite commonly lives the existence of a harmless saprophyte, it is difficult to understand why the same should not be true of Types I and II. Further research is needed on this point. Perhaps a fuller investigation of the miscellaneous collection known as "Type IV" will throw some light on the question. In the earlier days of meningococcus work, before the importance of serological differences was appreciated, a great many strains were found which did not respond to the particular sera with which they were tested and, on this account, might have been relegated to a miscellaneous scrap-heap similar to the American Type IV; but, now that more suitable sera have been obtained, the atypical residue which fails to respond to one or other of these has been greatly diminished. Similarly it may be possible to find pneumococcal sera which will rearrange and possibly link together the pneumococcal groups now known as I, II and IV. And, in this connection, another matter for consideration will be the possibility that bacterial antigen may be modified in the human tissues.

DIAGNOSTIC VALUE OF ABSORPTION OF AGGLUTININ.

COMPARISON BETWEEN INTESTINAL BACTERIA AND MENINGOCOCCI.

Certain investigators have claimed that between the two organisms *B. suipestifer* and *B. paratyphosus* (B), which are indistinguishable culturally and often agglutinate well with the same serum, a clear distinction can be brought out by resorting to the test for absorption of agglutinin. Their observations naturally raise the question whether the same method might not serve to distinguish between "non-contact" meningococci and strains of cerebro-spinal origin. I propose therefore first to state the evidence in support of the view that *B. suipestifer* and *B. paratyphosus* (B) are distinguishable by absorption of agglutinin and then to call attention to the results of this test when applied to meningococci obtained from different sources.

[1] Kolle u. Wassermann's *Handbuch der path. Mikroorg.*, 2nd Ed., IV. 498.

B. suipestifer and B. paratyphosus (B).

Boycott (1906)[1] in the course of an enquiry into the bacteriology of paratyphoid fever and the diagnostic value of serological tests, discussed the method of differentiation by absorption of agglutinin. As subsequent writers include Boycott amongst the investigators who are able to distinguish *B. paratyphosus* (B) from *B. Aertryck* (generally agreed to be identical with *B. suipestifer*) by the absorption method, it will be useful to select from Boycott's article the records of his experiments which have a bearing on this point.

(1) The serum of a rabbit immunised with *B. Aertryck* gave a titre of 1 : 2,000 for a strain of *Aertryck* and the same for a strain of *B. paratyphosus* (B). Absorption with *Aertryck* completely removed agglutinin for both strains; whereas absorption with *paratyphosus* (B) removed all agglutinin for itself but removed none of the agglutinin for *Aertryck*.

(2) The serum from a patient named "Barkley" was tested on two occasions. On the first, absorption with a strain of *paratyphosus* (B) removed all agglutinin both for this organism and for a strain of *Aertryck*, whilst absorption with *Aertryck* removed agglutinin for itself but not for *paratyphosus* (B). On the second occasion, when the titre of the Barkley serum was 1,000 for a strain of *Aertryck* and over 5,000 for a strain of *paratyphosus* (B), a single absorption with *Aertryck* removed the agglutinin for itself but did not affect the agglutinin for *B. paratyphosus* (B); absorption with *B. paratyphosus* (B) removed the agglutinin both for itself and for *Aertryck*, but only after treatment three times; the first and second doses of absorbing culture failed to remove agglutinin for either organism.

(3) The serum from a patient named "Valérie" lost its agglutinin for both *Aertryck* and *paratyphosus* (B) when absorbed with the latter organism; but, when absorbed with the former, the agglutinin was retained for *paratyphosus* (B) and lost for *Aertryck*.

The standard strain of *B. paratyphosus* (B) which Boycott used was "*Schottmüller B*, original strain (1901)"; and his *Aertryck* was a strain isolated by Prof. van Ermengem from an outbreak of food poisoning.

Bainbridge (1909)[2] used the absorption of agglutinin test for differentiating between *B. paratyphosus* (B) and the two indistinguishable organisms, *B. Aertryck* and *B. suipestifer*. The dilution of the serum used for absorption varied from 1 : 10 to 1 : 50 but was usually 1 : 20

[1] *Journ. of Hygiene*, VI. 33.
[2] *Journ. of Path. and Bact.*, XIII. 443.

or 1 : 40. It was found that differentiation was most clearly brought out "by comparing the agglutination limits of the serum for these bacilli after one or more absorptions with a moderate amount of bacilli."

A *B. Aertryck* serum (titre 1 : 5,000 for the homologous organism and also for a strain of *B. paratyphosus* (B)), when absorbed with *B. Aertryck*, failed to agglutinate either organism in 1 : 200; when the serum was treated with *B. paratyphosus* (B), the first absorption reduced the paratyphoid agglutination limit to 200 and the limit for *Aertryck* to 4,000, a second absorption brought down the former limit to below 100 and the latter to 2,000, a third absorption produced no further change.

A *B. paratyphosus* (B) serum (titre 1 : 5,000 for the homologous strain; 1 : 1,000 for a strain of *B. Aertryck* and the same for a strain of *B. suipestifer*), when absorbed with *B. paratyphosus* (B) failed to agglutinate all three organisms above 200; when the serum was treated with *B. Aertryck*, the first absorption reduced the paratyphoid agglutination limit to 4,000 and the limits for *Aertryck* and *suipestifer* to below 200; a second absorption reduced the paratyphoid limit to 2,000 and the limits for the other two organisms to below 100; a third absorption brought the paratyphoid limit down to 1,000.

A *B. suipestifer* serum (titre 1 : 10,000 for the homologous strain and also for a strain of *B. paratyphosus* (B)), when absorbed with *B. suipestifer*, failed to agglutinate either organism above 100; when absorbed with *B. paratyphosus* (B), agglutinin for this strain fell below 100 but agglutinin for *suipestifer* was retained at 10,000.

In the above experiments apparently the same three strains were used for producing the sera, for absorption, and for determining the titres of the sera before and after absorption.

Bainbridge has tabulated a further series of absorption experiments in which he used five sera (two *Aertryck*, two *paratyphosus* (B), and one *suipestifer*), absorbed each of these with an *Aertryck*, a *paratyphosus* (B), and a *suipestifer* strain, and determined the titre before and after absorption with three strains bearing the same designations (?actually the same strains). The results were in accordance with those obtained in the former series of experiments. Apparently some of the strains used in the second series, for producing the sera, for absorption, and for agglutination before and after absorption, were the same as those used in the first series.

Two of Bainbridge's standard strains, one *B. paratyphosus* (B) and one *Aertryck*, were the same as those used by Boycott. His two standard

suipestifer strains were "(*a*) the laboratory strain (Král); (*b*) a strain obtained from Prof. Wassermann."

O'Brien (1910)[1] isolated from an epizootic in guinea-pigs an organism belonging to the food-poisoning group. When tested with an *Aertryck* serum, this organism agglutinated up to full titre (1 : 5,000), as, also, did a strain of *B. paratyphosus* (B); absorption with *B. paratyphosus* (B) removed all agglutinin for itself but left agglutinin up to 1 : 2,000 for both *Aertryck* and the guinea-pig organism. When tested with a *para-typhosus* (B) serum, the guinea-pig organism agglutinated up to full titre (1 : 2,000), as, also, did a strain of *Aertryck*; absorption with *Aertryck* re-moved all agglutinin for itself and also for the guinea-pig organism, but left agglutinin up to 1 : 500, for *paratyphosus* (B).

Bainbridge and Dudfield (1911)[2] described an outbreak of acute gastro-enteritis caused by *B. paratyphosus* (B). Simple agglutination tests failed to discriminate between this organism and *suipestifer*, but the application of the absorption method brought out a sharp distinction in favour of the former bacillus.

Bainbridge and O'Brien (1911)[3] investigated the value of the ab-sorption method for grouping a certain number of strains which agglu-tinated well with both *paratyphosus* (B) and *suipestifer* sera.

The material they used consisted, in the first place, of certain stand-ard strains, all of which were well authenticated. These strains were:—
(*a*) Schottmüller's original strain of *B. paratyphosus* (B), which had previously been tested by Boycott in 1906 and by Bainbridge in 1909; (*b*) a *B. paratyphosus* (B) strain from McWeeney, apparently the one used by Bainbridge and Dudfield in 1911 in the investigation mentioned above; (*c*) a *suipestifer* strain designated "Laboratory (Král)," which had been used by Bainbridge in 1909 and by Bainbridge and Dudfield in 1911; (*d*) a *suipestifer* strain from Uhlenhuth; (*e*) a *suipestifer* strain from Wassermann which had been used by Bainbridge in 1909.

Secondly, 24 laboratory strains of the paratyphoid or food-poisoning group were collected from various sources and were compared with the five standard strains.

Referring to the technique of absorption experiments, the authors stated that it was possible by the addition of very large amounts of a heterologous bacillus to remove some of the homologous agglutinin from a serum, but they had not succeeded in removing all the homologous agglutinin in that way. "The difference between the amount of hetero-logous bacilli which must be added to serum to absorb only the hetero-

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[1] *Journ. of Hygiene*, x. 231.　　　[2] *Ibid.* xi. 24.　　　[3] *Ibid.* x. 68.

logous agglutinin, leaving the homologous agglutinin intact, and that necessary to absorb much of the homologous agglutinin, is so large that errors cannot occur if reasonable care is taken." For example, they used 2 c.c. of a 1 : 10 dilution of *suipestifer* serum (titre, 1 : 20,000 for the homologous strain, 1 : 5,000 for a strain of *B. paratyphosus* (B)); absorbed with two agar slopes of *paratyphosus* (B), its agglutination limit was reduced to below 100 for *paratyphosus* but remained at 20,000 for *suipestifer*, and the latter limit remained unaltered when absorption was made with eight agar slopes. The authors observed that it was preferable to measure the maximum titre of agglutination with the serum after absorption rather than merely to observe the agglutination at one or two dilutions.

The authors have recorded in full the essential details of their laboratory work; as the results were uniform, a brief summary will suffice. The absorption tests divided their five standard strains into (1) (*a*) and (*b*), which conformed to their *paratyphosus* standard, and (2) (*c*), (*d*), and (*e*), which conformed to their *suipestifer* standard. Absorption tests also divided their 24 additional strains into (1) those conforming to their *paratyphosus* standard and (2) those conforming to their *suipestifer* standard, with the following exceptions—one (No. 19) agglutinated with unabsorbed *suipestifer* serum up to 10,000, and the titre after absorption with *paratyphosus* fell to below 100, but the same organism gave 5,000 with unabsorbed *paratyphosus* serum and after absorption with *suipestifer* the titre fell to below 200; of two other strains, one (No. 16) was a poor agglutinator and the other (No. 20) was practically inagglutinable with the two standard sera.

Meningococci.

For the details of the work on this subject I must refer to the reports by Dr Griffith, Dr Scott, and Captain Ponder.

Dr Griffith (p. 129) has found that 62 of his 86 naso-pharyngeal strains agglutinate up to 400 or higher with one or more of his Group II sera prepared from spinal strains. He has tested the absorptive capacity of 33 out of these 62 strains, and has found that they all exhaust the homologous agglutinin from one or more of his spinal sera. This result, he considers, is sufficient to justify the conclusion that the remainder of these 62 strains would be found to absorb the homologous agglutinin from some Group II spinal serum. Of his remaining 24 strains, some, as he has shown in detail on pp. 161–4, are related in absorptive capacity to Group I strains of spinal origin.

Dr Scott has found (p. 246) that 58 of his 71 naso-pharyngeal strains afford proof of complete serological identity (including identification by the absorption test) with known pathogenic strains.

Captain Ponder (p. 280) has not tested all his strains by the absorption method, but he has taken the cultures from his last series of 100 swabs, all obtained from healthy workpeople, and has submitted to the absorption test all those which were like meningococci in simple agglutination. He only employed one serum for this purpose. He found that nine of his strains absorbed agglutinin as well as the homologous coccus, and four others absorbed it partially. On applying the absorption test to another batch of strains he obtained similar results.

Validity of Differentiation by Absorption of Agglutinin.

The results of the work on the meningococcus suffice to show that absorption of agglutinin tests do not separate "non-contact" from cerebro-spinal meningococci in the way in which such tests divided the strains of *B. paratyphosus* (B) and *B. suipestifer* which were investigated by Bainbridge and O'Brien. This lack of correspondence raises several problems which require consideration, the first question being whether the work of Bainbridge and O'Brien justifies a general statement that organisms giving the cultural reactions common to the large group of which *B. paratyphosus* (B) and *B. suipestifer* are members can be differentiated by absorption of agglutinin. This statement has been disputed by certain German pathologists, on grounds which are given in the following summary.

Objections.

Uhlenhuth, Hübener, Xylander, and Bohtz (1909)[1] maintained that *B. paratyphosus* (B) and the hog-cholera group of organisms could not be classified into separate groups either culturally or by serological tests. In this connection they discussed the contention that, though indistinguishable culturally and by simple agglutination tests, *paratyphosus* (B) and *suipestifer* were clearly separable by the adoption of Castellani's principle of differentiation by the absorption of agglutinin. This, they found, was not the case. They admitted that clear differences might be apparent if reliance were placed on a single strain as representative of each alleged group of organisms; but, when a large number of strains were used, the results of the absorption tests were so irregular that a demarcation into distinct groups became impossible. This irregularity

[1] *Arb. a. d. Kaiserlich. Gesundheitsamte*, xxx. 292.

they attributed to individual differences in the "receptor apparatus" of different strains. They based their conclusions on the results of absorption tests with a very large number of different strains and, in the present article, have taken one series of experiments as an example and tabulated their results. The table is too long to reproduce here, but it will suffice to quote some of the essential details of the experiments which it records.

Their method of conducting the tests was as follows: a rabbit serum (titre 1 : 5,000) was prepared from a human *paratyphosus* (B) strain named "Hellwig," and was diluted to 1 in 500. To 100 c.c. of this dilution was added, in the case of each strain used for absorption, the 24 hours' growth obtained on 20 agar tubes. The mixture was incubated at 37° C. for two hours, and then centrifuged until completely clear fluid was obtained. For testing the absorbed serum as to its remaining agglutinating power (dilution 1 : 500), 1 loopful of 24 hours' culture was used to 1 c.c. of fluid; the mixture was incubated at 37° C. for one hour and then kept at room temperature for 24 hours. The same procedure was adopted in each test.

When the homologous strain, "Hellwig," was used for absorption, it removed the agglutinin for itself and for the 19 other human *paratyphosus* (B) strains which were tested.

But when other strains were used for absorption of this serum uniformity of results was no longer obtained.

Absorption with "Eb.," one of the above 20 human paratyphoid strains, removed agglutinin for itself but not for "Hellwig," and with the remaining 18 strains the results were irregular, agglutinin being removed for eight, but retained for eight others, whilst with the last two the result of the test was doubtful.

Absorption with "England," another of the above 20 strains, removed agglutinin for itself, for "Eb.," for "Hellwig," and for 12 other of these strains, but failed to remove agglutinin from the remaining five, including one which gave the opposite result when the serum was absorbed with "Eb."

The "Hellwig" paratyphoid serum was then absorbed with certain strains of *suipestifer* obtained from pigs, using the same quantities of culture and the same technique in every respect as in the former experiments. The first *suipestifer* strain removed agglutinin for 18 out of 27 *suipestifer* strains, all isolated from pigs; but it also removed the agglutinin for "Hellwig" and for 12 other of the 20 human *paratyphosus* (B) strains. It failed to remove the agglutinin for "Eb.," for "England,"

and for four other strains. With the one remaining strain the result was doubtful.

A second *suipestifer* strain, from a normal pig, when used for absorption of the same paratyphoid serum, removed the agglutinin for 21 out of the 27 *suipestifer* strains; but it also removed the agglutinin for "Hellwig" and for 11 other of the 20 human *paratyphosus* (B) strains. It failed to remove the agglutinin for the remaining eight, including "Eb." and "England."

Similar irregularities in results were obtained when the serum was absorbed with other strains, *e.g.,* a strain obtained from a case of food poisoning and one isolated from a sausage. These need not be quoted in detail, as the above data suffice to support the authors' statement that the method was found to be unreliable for diagnostic purposes. Of the five absorbing strains about which I have given details, only one gave unequivocal results; and that was the one used to produce the immune serum. The other two paratyphoid (B) strains used for absorption failed to absorb agglutinin for several paratyphoid (B) strains; and the two *suipestifer* strains used for absorption, which, according to the absorption theory, should have left the paratyphoid agglutinin untouched, removed this agglutinin for more than half of the paratyphoid strains.

Are the Objections Valid?

The importance of the laboratory data, quoted above, which were recorded by Uhlenhuth and his co-workers in 1909, lies in the fact that, under identical conditions of experiment, the individuality of different strains comes out very strongly and makes its appearance in such irregular fashion that no basis is provided for a subdivision of these strains into distinct groups.

But, in the absence of fuller particulars, these results cannot be regarded as a conclusive proof that differentiation of these organisms by the adoption of Castellani's principle is impossible. No information is given as to the highest dilution in which agglutination was obtained after absorption, and there is no evidence that the quantity of culture used for absorption was the amount most favourable for enabling a group distinction to make its appearance.

For example, the paratyphoid serum (titre, 1 : 5,000) was absorbed with the paratyphoid strain "Eb." and it was found that the absorbed serum still agglutinated eight paratyphoid strains at 1 : 500. But this is a very incomplete statement of the relationship of "Eb." towards

these eight strains. Presumably, these eight strains agglutinated with the unabsorbed serum as well, or nearly as well, as the homologous strain. Did absorption with "Eb." leave their agglutination limit unaffected or only slightly affected, or did it bring that limit down somewhere to the neighbourhood of 500? As no answer to these questions is given, it is impossible to exclude the latter alternative. Furthermore, since no evidence is given to the contrary, one cannot exclude the possibility that a much smaller quantity of "Eb." culture would have been as effective, or nearly as effective, in bringing about a marked reduction of agglutinin for all the 20 paratyphoid strains.

Again, when a *suipestifer* strain was used for absorption, similar questions arise concerning its failure to remove agglutinin (at 1 : 500) for 9 out of 27 *suipestifer* strains. Possibly it effected a marked reduction of agglutinin for these nine strains; and possibly a much smaller quantity of absorbing culture would have produced very similar results on all 27 strains.

Moreover, in the absorptions with *suipestifer* strains, the objection has not been met that the use of much smaller quantities of absorbing culture might have produced relatively little loss of agglutinin for paratyphoid strains but, at the same time, well marked loss for *suipestifer* strains.

It will be noted also that the serum used for absorption was very dilute (1 : 500), and therefore more readily affected than more concentrated sera.

In considering the work of Bainbridge and O'Brien it must at once be recognised that they have investigated a considerable number of strains and, by means of the absorption method, have succeeded in almost every instance in allocating each to one of two groups, according as it conforms (a) to their *paratyphosus* standard or (b) to their *suipestifer* standard.

At first sight the fact that a considerable number of strains was used seems to dispose of Uhlenhuth's objection that division into groups is only possible when attention is confined to single strains as representative of each alleged group. The strains used for testing against the standard organisms were isolated from human or animal material sent to several different laboratories, and were therefore derived from several different and independent human or animal sources; and in most cases, presumably, it had been established in the laboratories providing these strains that they were typical representatives of the paratyphoid (B) and food-poisoning group of organisms, and were therefore suitable for

submission to the absorption test. But here an important laboratory point arises. As a matter of routine diagnosis the strains must have been identified by testing their agglutinability with standard sera, and probably these standards were established either by the original strains regarded as representative of this group of organisms (*Schottmüller* (B) and Van Ermengem's *B. Aertryck*) or by strains proved to be identical with these. Many investigators have found that a "typical strain," *i.e.* one which conforms to their standards, can be differentiated by simple agglutination alone, since, when tested with good sera, its agglutination limit is much higher for *paratyphosus* serum than for *suipestifer* serum or *vice versa*. It therefore seems fair to raise the question whether the majority of the strains sent to Bainbridge and O'Brien had already been selected as "typical," in the sense defined above, or whether they were random samples and truly representative of the range of variation of *B. paratyphosus* (B) and of *B. suipestifer* which may occur in nature. Bainbridge and O'Brien give no information on this point. They have, however, found that, when their 24 strains were tested with sera prepared by injection of living cultures, before resorting to absorption "some indication was revealed of the existence of two types of bacilli." Referring to their tabulated record of these experiments, it is seen that two of their 24 strains may be omitted as being poor agglutinators with both the *suipestifer* and the *paratyphosus* serum, and that each of the remaining 22 showed a clear difference between its agglutination limit with the one serum and its limit with the other. This difference was never less than 2 :1 (*e.g.* 20,000 as against 10,000 or 10,000 as against 5,000) and occasionally it was much greater. These results are strong indication of initial conformity to standard; and it will be found that the diagnosis thus indicated by agglutination alone is confirmed in 21 out of the 22 strains by the authors' subsequent absorption tests. The exceptional case is No. 19 which on simple agglutination, reached 10,000 with *suipestifer* serum as against 2,000 with *paratyphosus* serum; the absorption results, however, are ambiguous, as absorption of *suipestifer* serum with *paratyphosus* reduced the agglutinin for this strain from 10,000 to below 100, whilst absorption of *paratyphosus* serum with *suipestifer* also effected a marked reduction in agglutinin for the strain (from 5,000 to below 200).

On this view, the fact that all the strains investigated had been well authenticated may have been a disadvantage, because it may mean that they had been selected as "typical" owing to their conformity with one or other of two well-known serological criteria. If this was the case,

Uhlenhuth's objection against the absorption method has not been refuted. On the other hand, supporters of the absorption theory may regard Uhlenhuth's laboratory data as inconclusive. It will be best, therefore, to leave the question as still unsettled. Evidently these intestinal organisms show a tendency to serological grouping, just as meningococci do, and they may possibly be capable of subdivision into two large groups supplemented by a number of smaller ones. But, as the groups have not yet been fully worked out, one cannot take it for granted that the members of some of them will be exclusively "suipestifer" and the members of others exclusively "paratyphoid."

These considerations of laboratory detail are important because they have a direct bearing on a general question of bacteriological classification, which concerns the meningococcus and many other organisms, in addition to *paratyphosus* (B) and *suipestifer*[1].

How much stress ought to be laid on individual peculiarities of particular strains? There can be no doubt that in many, if not in all, widely distributed groups of organisms such peculiarities do exist; and this fact is usually brought into prominence whenever the stimulus of research leads to minute examination of a large number of organisms belonging to the same class. Then the difficulty of standardisation arises. The obvious course is to begin with a particular strain as a provisional standard and see how many other strains coincide more or less completely with this, in agglutinating with and absorbing agglutinin from the serum produced by the standard strain. If there remains a residue of aberrant strains, a second provisional standard is selected from these, and it is ascertained how much of the residue can be grouped under this second standard. To the strains, if any, which refuse to fall into the second group a similar process is applied, and so on, until all the strains are accounted for.

This method is unimpeachable if it is recognised as being no more than a preliminary orientation; but it is fallacious if it is taken as providing a final classification. Suppose, for example, the orientation method gave three groups, the respective standard strains being A, B, and C. One cannot take it for granted that all the strains in Group I are identical with A in agglutinogenic and absorptive capacities, nor that the same holds good for Groups II and III; but without such identity

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[1] It is in view of this general question that I have discussed the significance of absorption experiments with food-poisoning organisms; the question of what is the best way to classify these particular organisms would involve consideration of many matters, in addition to absorption experiments, and does not come within the scope of this report.

the grouping would not be justified, since the selection of other strains as standards would then give different groups. The true standard is that which represents what exists in nature, and therefore must recognise such individual differences as occur; a standard which ignores these would be arbitrary, artificial, and not truly representative.

To put the same considerations in a more technical form, absorption is supposed to aid classification by bringing out a distinction between specific and non-specific agglutinin; the value of the absorption method must therefore be discussed in relation to theories as to the specificity of agglutinin.

PRINCIPLES DETERMINING DIFFERENTIATION OF AGGLUTININS.

I. Bacteria of Different Species.

If the agglutinin for each species were quite distinct from the agglutinins for the rest, the matter would be very simple. For example, a serum containing the agglutinins a, b, c would be due to mixed infection with the three species A, B, and C; and each species, as shown by Castellani, would absorb its own agglutinin from the serum, leaving the other agglutinins intact.

But it very often happens that there is a partial overlapping, to greater or less degree, of agglutinins produced by bacteria which are recognised, from their general biological characteristics, as belonging to distinct species. On Durham's hypothesis, this fact would be explained by assuming that agglutinin consists of several different components. Thus:

Species A may produce agglutinin $a\,b\,c\,d\,e$.
Species B may produce agglutinin $a\,b\,f\,g\,h$.
Species C may produce agglutinin $b\,c\,i\,k\,l$.

Accepting this hypothesis, provisionally, the specific agglutinins would be contained amongst the components $d\,e$, $f\,g\,h$, and $i\,k\,l$, respectively, whilst the corresponding non-specific agglutinins would be represented by $a\,b\,c$, $a\,b$, $b\,c$. This distinction would again be demonstrable by Castellani's method; e.g. absorption of an A serum with a B strain would remove all agglutinin for species B but would leave agglutinin practically intact for species A.

This method has often been applied as an aid to diagnosis. For example, an unknown culture X is agglutinated both with serum A and serum B. Serum A is then absorbed with culture X and the absorbed

serum is tested upon a known strain of species A. If it is found that X has removed the agglutinin for the known strain, X is regarded as belonging to species A. If this is not the case, a similar test with B serum and a known B strain may show that X belongs to species B.

The validity of this method is widely recognised in cases where a positive result is obtained, *i.e.* when the unknown strain removes specific agglutinin for either A or B.

It is to be noted, however, that if the result is negative, *i.e.* neither strain A nor strain B fails to agglutinate with the serum absorbed by X, it is not justifiable to conclude that X belongs to a third species different from both species A and species B, because a particular strain does not necessarily absorb agglutinin for all members of the same species. In such a case the absorption test would give no information of diagnostic value.

This important fact that strains of the same species may differ from each other in absorptive capacity is well illustrated by the work of Meinicke, Jaffé and Flemming (1906)[1]. They tested the absorptive capacities of 47 cholera strains which were all typical and all agglutinated well, and about equally, with a standard cholera serum; this serum, which had been prepared from one strain, had no effect on "cholera-like" vibrios. Marked differences were found. Some strains absorbed agglutinin for the whole or the majority of the 47 strains; but others only removed agglutinin for a relatively small number, and in this respect they exhibited a selective action, *i.e.* the strains picked out by some absorbing strains were not the same as the strains picked out by others. This selective action was qualitative and not merely quantitative, because a strain which only removed agglutinin for a few strains could not be made to remove agglutinin for more by repeating the absorption. According to absorptive capacity, their 47 strains might be divided, roughly, into five different groups, but the demarcation of these groups was not always sharply defined, and within some of the groups a subdivision might be made. The authors thought that if the number of their strains had been larger the number of groups would probably have increased, but they fully recognised that attempted grouping of cholera strains in accordance with absorptive capacity would be devoid of practical interest.

This irregularity of absorptive capacity is particularly significant, because the cholera vibrio is remarkably specific in agglutinability and agglutinogenic capacity. With other species which are less uniform in

[1] Ueber die Bindungsverhältnisse der Cholera-vibrionen. *Zeitschr. f. Hyg.*, LII. 416.

the two latter respects, it is still less likely that diagnostic significance can be attached to irregularities in capacity for absorption.

II. Possible Sub-Groups of one Species.

Whilst the absorption method often gives positive results in determining the species to which an organism belongs, its applicability to the sub-grouping of members of one species, which coincide in simple agglutinability, is another matter. In the former case, its utility consists in eliminating the ambiguity caused by the overlapping of two different sera which contain heterologous as well as specific agglutinin. In the latter case, it is not a question of distinguishing specific from heterologous or accidental agglutinin, but of emphasising those characteristics of the sub-groups which are not common to the species as a whole. Thus, borrowing Durham's conception of the multiple components of agglutinin, the sub-groups of a species may behave as follows:

> (a) Group I may produce agglutinin $a\,b\,c\,d\,e$.
> Group II may produce agglutinin $d\,e\,f\,g\,h$.
> Group III may produce agglutinin $d\,e\,i\,k\,l$.

The special characteristics of each group would be contained amongst the components $a\,b\,c$, $f\,g\,h$, $i\,k\,l$; the agglutinin common to the whole species would be $d\,e$. This distinction, as in dealing with organisms of different species, would be brought out by the absorption method, but the nature of the distinction would be very different. Here, $d\,e$, which is common to the three groups, is essential; it is not heterologous and cannot be eliminated as unimportant. Its presence is in no way comparable to the accidental overlapping by $a\,b\,c$, $a\,b$, and $b\,c$, in the three different specific sera which I have figured above (p. 89).

I wish to emphasise this last point, because much confusion has been caused by loose usage of the terms "specific" and "non-specific." Admittedly, bacteriological "species" are more or less ill-defined, but there need be no practical difficulty on that score. For example, gonococci and meningococci are quite sufficiently different to be called different species; differentiation, by absorption, of a gonococcus and a meningococcus which are agglutinated by the same serum is a specific differentiation. On the other hand, cholera vibrios are all sufficiently alike to be included in one species, and the same may be said for typhoid bacilli; differentiation, by absorption, of two cholera or two typhoid strains which agglutinated with the same serum would be a minor

distinction, possibly affording a basis for sub-grouping, but not invalidating the evidence of specific relationship afforded by the simple agglutination test.

I have taken the simplest case first, where the sub-groups possess some agglutinins in common and thereby show their relationship to the species as a whole. Here, as Meinicke and his colleagues have shown, sub-grouping is possible by means of the absorption test, but in their experience with strains of cholera it seemed to be of minor importance and in no way corresponded with differences of virulence. Instead of providing a useful basis of classification, the differences in absorptive capacity which they demonstrated proved, in their opinion, that "the Castellani test could no longer be regarded as an infallible criterion."

Then there is another possibility, in the sub-grouping of some species of bacteria, where the sub-grouping is equally simple but of a different kind, being based on the absence of serological affinity between the groups, as shown by simple agglutination tests alone. Thus:

> (b)   Group I may produce agglutinin *a b c*.
>   Group II may produce agglutinin *f g h*.
>   Group III may produce agglutinin *i k l*.

According to the American investigators referred to above (pp. 74—77) the pneumococcus, excluding the more or less distinct species *Pneumococcus* or *Streptococcus mucosus*, affords an example of such a species.

In such a method of grouping, as there is no overlapping of agglutinins, resort to the absorption test is not required.

But more commonly, particularly when a large number of strains is examined, the sub-grouping of a species is too complex a task to adapt itself to either of the simple schemes (*a*) and (*b*), and the question arises as to how far absorption is an aid to classification in these cases.

The complexity is due to the fact that the members of each proposed group are not identical in every respect. It is found, for example, that some proposed members of Groups I and II in scheme (*b*) produce, in addition to their special agglutinins *a b c, f g h*, some further agglutinin, *d* or *d e*, which is common to both groups, as in scheme (*a*). Again, it may be found that other members of a group, *e.g.* Group I in scheme (*b*), produce less than their special agglutinins *a b c*; some strains, as shown by absorption of the sera produced by them, may possess only the *a b* antigen, or only *a c*, or *b c*, or *a*, *b*, or *c*. Hence the absorption test, at first called in to justify the groups originally postulated, would make the further demand that these groups must again be subdivided.

Put schematically, the position would be:

(c)   Members of Group I may combine with one or more or all of the agglutinins a, b, c, d, e.

Members of Group II, similarly, with d, e, f, g, h.

Members of Group III, similarly, with d, e,......i, k, l.

Thus the agglutinin for the species as a whole would comprise all the components a to l, and each member would be identified by one or more of these components. If the individual members could be grouped as in the above scheme, i.e. sometimes with partial but never with complete overlapping of the main groups, grouping would be possible but the main groups would need further subdivision.

But here, as in the simpler case of the cholera vibrio, it is difficult to understand what diagnostic significance can be attached to a classification based on irregularities of absorptive capacity.

III. A Species not divisible into Sub-Groups.

It is not uncommon to meet with strains of bacteria possessing individual peculiarities which simply have to be recognised as such. This is no bar to classification, provided that the characteristic agglutinin for the species or for the sub-group remains demonstrable for every strain. But it is also possible that the members of a species may evince individuality of such a nature that they are not amenable to classification into groups.

As contrasted with Scheme (c), it would be impossible in this case to assign certain agglutinins as the exclusive property of one or other of the postulated main groups. Thus:

(d)   The complete agglutinins of the species $= a$, b, c, d, e, f, g, h, i, k, l. Individual members may combine with only one, or more, or all of these.

But the last scheme would not be a satisfactory representation of the species because it does not bring into expression the underlying unity of specific antigen upon which individual variations are superimposed. It would probably be more accurate to assume that specificity is based upon a common constitution of the protein molecule, to which a variable receptor apparatus is attached. Thus the agglutinin complex of each strain will possess a common characteristic, which may be designated A, and this, in the case of different strains, will be associated

irregularly with minor elements, b, c, d, e, etc., not individually repre-
sentative of the species as a whole. For example:

(*e*) Strain (1) may produce agglutinin:—

          ,,   (2) ,,   ,,   ,,

          ,,   (3) ,,   ,,   ,,

```
 b\        /c
    \     /
     \ A /
     / \ 
 d /     \ e
 f \     / g
     \ A /
     / \ 
 h /     \ i
 k \     / l
     \ A /
     / \ 
 m /     \ n
```
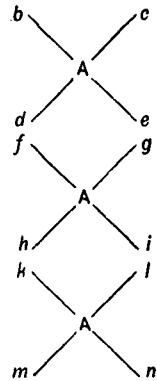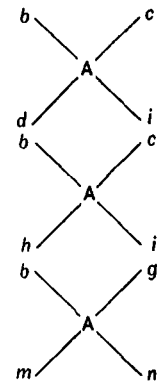
With these three strains A is shown as linked to four elements, and each
of these is different for each strain. But other and less sharply contrasted
expressions of individuality may occur, *e.g.*:

(*f*) Strain (4) may produce agglutinin:—

          ,,   (5) ,,   ,,   ,,

          ,,   (6) ,,   ,,   ;,

```
 b\        /c
    \     /
     \ A /
     / \ 
 d /     \ i
 b \     / c
     \ A /
     / \ 
 h /     \ i
 b \     / g
     \ A /
     / \ 
 m /     \ n
```

Strains (4), (5), (6) differ less from strain (1) than do strains (2) and (3);
and it might be found that the agglutinin produced by strain (1), and
therefore combining with strains identical with this, failed to combine
with strains (2) and (3) but was able to unite with less divergent strains
such as (4) and, though perhaps not equally well, with strains such as
(5) and (6).

Similarly with agglutinin produced by strain (2). This might not
affect strains (1) and (3) but it might interact with strains less divergent

from, though not identical with, the homologous strain. And the like would hold with strain (3) agglutinin.

Strains (1), (2) and (3) are taken as examples of strains sharply differing from each other, as shown by their respective elements *b c d e*, *f g h i*, *k l m n*. Obviously, the number of such strains may be very large: *e.g.* a fourth may be associated with *b f m g*, a fifth with *c h i k*, a sixth with *d e g l*, and so on.   And, as illustrated above, each of these strains may produce agglutinin which will pick out not only strains identical with the homologous but also strains in which the difference from the homologous does not amount to a sharp contrast.

Hence, on testing the strains of this species with a large number of monovalent sera, the broad result would be a reiterated and irregular demonstration of resemblances and differences between different strains. The resemblances would be due to the fact that all the strains belong to the same species; the differences would be the expression of individual peculiarities. Scientific grouping would be impossible because, unlike the species discussed in Section II, the species now under consideration contains an indefinitely large number of strains which differ in qualitative capacity for producing and combining with agglutinin.

IV. The Question whether a Given Species is or is not divisible into Sub-Groups.

It follows from the above considerations that if the strains belonging to the species fall into distinct groups, without cross-division, when tested with an extensive number of monovalent sera, grouping is indicated; but if such tests produce marked cross-division, grouping is not justifiable. For example, sera I, II and III may apparently divide meningococcal strains into three corresponding groups; but if sera prepared from other members of these groups invalidate this distinction, it may be inferred that the three groups do not represent an accurate subdivision of meningococcus antigen into three distinct and separable types.

V. General Remarks.

In determining whether a doubtful organism belongs to one or other of certain different species, the absorption method is certainly useful sometimes, as a supplementary test, provided that the result is positive, *i.e.* that evidence of specific absorption is obtained.

The method has been applied clinically, by testing the patient's serum with bacteria of known species, and has been the means of iden-

tifying the bacterial cause of the disease, when the results of simple agglutination were ambiguous, and also of demonstrating cases of mixed infection. There are certain fallacies to guard against, as Paltauf has pointed out. The infection may really be due to an unsuspected organism, which has produced in the patient some heterologous agglutinin for one of the organisms under suspicion. For example, the diagnosis may be thought to lie between typhoid, paratyphoid, and Gärtner infection, and it may be found that the agglutinin which acts on these three organisms is absorbed by the typhoid bacillus; but the case may really be an infection with *B. proteus*, which has produced some heterologous agglutinin for the typhoid bacillus. But instances such as this do not detract from the fact that the method is admittedly useful. Similarly, when the organism isolated from the patient gives ambiguous agglutination results with laboratory sera representative of different species, a positive absorption test may help to settle the diagnosis.

On the other hand, a strain cannot be excluded from a species because the result of the absorption test is negative. Such cases, where the diagnosis must be determined by the general biological characters of the organism taken as a whole, serve as a useful reminder that serological reactions are not always infallible and do not necessarily play the decisive part in determining classification.

The sub-grouping of a species may turn out to be an easy or a difficult matter. This will depend partly on the homogeneity or the irregularity of specific antigen and partly on the use of a small or a large number of strains for the demonstration of agglutinogenic and combining capacities. It may be found, to begin with, that simple agglutination alone divides the strains into groups with no overlapping, as in Scheme (b). If the addition of more strains and more sera confirms this classification, well and good. If, however, it is now found that there is some overlapping, as in Scheme (a), resort will be made to absorption. This, possibly, will still support the original grouping, which will now be an amalgamation of Scheme (a) and Scheme (b). It may turn out, however, that in the enlarged series further differences of antigen are found between members of the same group, as in Scheme (c). Even now it may still be possible to provide a theoretical justification for the original groups, supplemented by a subdivision of each, provided that there is no more than a partial identity between the postulated complete antigen of each of the three main groups; though it hardly seems likely that such an elaborate classification would be of diagnostic value. At this stage, where each main group is so elastic that its margin of separation from the

others is small, one begins to raise the question whether the adopted system of grouping has not turned out to be artificial and arbitrary, and whether the species under consideration is really distinguishable from one which is not amenable to sub-grouping, as in Schemes (d), (e) and (f). One's decision will naturally be influenced by observing whether the proposed grouping does or does not involve the confusion of cross-division.

DIFFERENTIATION OF AGGLUTININS IN RELATION TO THEORIES OF IMMUNITY.

For the above discussion I have taken as the starting-point Durham's hypothesis of a multiplicity of agglutinins in monovalent immune sera, because his views are well known and have obtained wide, though not universal, acceptance. Like all other explanations of immunity which are based on Ehrlich's principles, Durham's theory postulates an indefinite or unlimited number of unknown chemical components, and on this ground it may be open to the objection, which has been raised against many of Ehrlich's postulates by the opposing school of Bordet, that this free coinage of hypothetical chemical entities is merely a restatement of laboratory data in terms of the unknown, a resort, in fact, to the fallacious method of exposition known as *ignotum per ignotius*.

Perhaps there is some element of truth in this objection. For example, one might be tempted to begin by postulating that a particular agglutinin contained the components *a*, *b*, *c*; when laboratory facts came to light which showed that this explanation was insufficient, another component, *d*, might be tacked on to it; and this, when further occasion required, might be supplemented by *e*, *f*, *g*, etc. .Obviously such postulates would be no real explanation but merely a redundant way of saying that the phenomena of agglutination are complex and of unknown nature.

At the same time one must recognise the importance of Ehrlich's general principle that the specificity of agglutinins is determined by their precise chemical constitutions, though these are too imperfectly understood to be expressed in the rational formulae of the organic chemist. This chemical conception, though unfortunately vague, cannot be ignored, because specificity cannot be explained on purely physical grounds; it must, however, be supplemented by the equally important conceptions of immunity which are based on experimental physics and the properties of colloids, since these principles, whilst not explaining specificity, play an essential part in agglutination.

The difficulty of Ehrlich's theory, it appears to me, is twofold; it is exclusively chemical and much weakened by controversial antagonism which refuses to recognise

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the value of physical theories; and the chemical conceptions follow too closely analogies derived from the study of aniline dyes and experimental pharmacology. One needs a theory which will link up the chemical and the physical sides of the problem, instead of making them appear irreconcilable; and it is doubtful if the groups and side-chains which determine the properties of dyes and drugs are analogous to the differences in chemical structure which determine the differences of various antigens and antibodies. The fact that neither a purely chemical nor a purely physical theory will suffice is emphasised by Paltauf (1913), who has reviewed with remarkable impartiality conflicting theories as to the nature of agglutination[1]. He concludes:—"Agglutination (and precipitation) is a genuine colloid reaction, but for the specificity of this reaction the chemical constitution of the interacting colloids is responsible. This determines the formation of the complex combinations which are associated with alteration of the conditions of solubility, and, by altering surface tension, determine the process of clumping."

The problem has been simplified by Bordet's conception, which has now been accepted by the majority of observers, that the agglutination reaction takes place in two phases, the first being "the period of impression" and the second "agglutination properly so called."

The result of the first phase is that a change takes place in the colloidal nature of the bacteria; this change involves, according to some physicists, with whom Paltauf appears inclined to agree, a conversion from the emulsoid into the suspensoid form. Then, coming to the second phase, there is an interaction between two colloids (bacteria and serum) in the presence of salts, with consequent agglutination, if this is compatible with the physical condition of the bacterial colloid.

This view of Bordet's, now well established, serves to clear the ground and enables one to concentrate attention on the first phase, in which the specific interaction between antigen and antibody takes place.

Here chemical constitution must play a part, as it is difficult to see how physical conditions alone can determine specificity. The postulate of "specific absorption" merely postpones the difficulty, as the specificity of the interaction must depend on chemical constitution. Durham's conception of antigen and antibody as possessing a multiplicity of different chemical groups is at least chemical; and on this ground, it might be urged, it ought to be accepted until something better can be found to replace it. One way of emendation, advocated by some bacteriologists, is to introduce the conception of differences in "avidity" on the part of particular antigens. On this assumption, two conditions are necessary for the union between a particular antigen and a particular antibody, (1) appropriate chemical constitution and (2) the character termed "avidity." (1) is not necessarily accompanied by (2); when it

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[1] Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen*, 2nd Ed., II. pt 1, pp. 483–654.

is not, union with antibody does not take place. The general conception of specific antigen and antibody as containing a large number of different chemical groups is retained.

I have already referred (p. 90) to the observations of Meinicke and his colleagues on differences in the absorptive capacities of cholera vibrios. Following Durham's hypothesis, one might endeavour to explain these results as in scheme (a) on p. 91, viz.

Group I may contain antigens a, b, c, d, e.
,,   II may contain antigens d, e, f, g, h.
,,   III may contain antigens d, e...i, k, l.

The strains agree in agglutinability because they all possess the antigens d, e, but they differ in absorptive capacity owing to the presence or absence of certain other antigens; e.g., absorption with a Group I strain will remove all agglutinin for members of this group, but not for strains containing any of the antigens f...l.

But against this explanation there are two objections. (1) The strains agree in agglutinability not merely with the same serum but with different sera; e.g., though Group I and Group II do not absorb for each other, a Group I strain will produce a serum which agglutinates Group II strains and *vice versa*. (2) Under identical conditions of experiment, different strains absorb for themselves the same amount of agglutinin from different sera. (1) indicates that the different groups have not been found to differ qualitatively, i.e., as regards the presence or absence of particular antigens; and (2) shows that they have not been found to differ quantitatively as regards the amount of particular antigens possessed, i.e., it cannot be postulated that Group I strains possess all the antigens of the three groups (as shown by agglutinogenic capacity), but possess certain of these antigens in much smaller amounts than the other groups (as shown by lack of absorptive capacity); for if this were the case the amount of agglutinin which a strain absorbed for itself, under the same conditions of experiment, would differ according to the group-membership of the strain used for preparing the serum.

Meinicke and his colleagues meet this difficulty by postulating differences in "avidity." All their strains, they argue, possess all the cholera antigens, say a to m, but *in vitro* some of these elements lack "avidity" (capacity for combining with the corresponding agglutinin); thus, for one strain the only "avid" elements may be a, b, d, k, m; for another the elements, c, e, f, n, and so on. *In vivo*, however, the greater disintegration which takes place in the animal tissues releases all the antigens in an active condition, and consequently a serum is produced which contains every representative agglutinin.

This postulate of "differences in avidity" amongst different components of an antigen appears to me unnecessarily complex. I think the element of truth it may contain would be better expressed by a broad recognition of the fact that the combining capacities of antigen as a whole are affected by its particular chemico-physical condition.

Meinicke's experiments may then be regarded as showing that cholera antigen is one and the same, in essential chemical structure, and so is cholera antibody, but

minor differences exist *in vitro* and are brought out by the absorption test. This fact may be explained, not by representing a specific antigen as consisting of several different components coexisting side by side, but by regarding it as a chemical substance which may exist in one or other of several different chemico-physical phases, demonstrable by test-tube experiments. And the same conception would apply to antibody. Thus, when cholera culture and antiserum are brought together, the amount of culture being sufficient to remove the whole of the agglutinin with which it is capable of combining, the cholera antibody is affected as a whole by this interaction and any that is left uncombined settles down into equilibrium by a process of readjustment, involving such changes as constitute a new chemico-physical phase. The residual agglutinin, owing to its change of state, can only combine with such cholera strains as are in a different chemico-physical phase from the original absorbing strain.

This conception is not inconsistent with Paltauf's view that "according to the nature of the molecule as a whole, certain properties may vary, although the specific reacting group remains the same."

It is a conception which implies varying complexity in the structure of one and the same specific substance; it differs from the conceptions of Meinicke, Durham and others, which imply that specific substance is not one but multiple, and that each component is separable from the rest.

At this point it will be useful to give more definite significance to the term "chemico-physical phase" by reference to experimental facts. Apart from changes affecting only the second stage of the agglutination reaction, *i.e.*, changes in agglutinability without changes in absorptive capacity, variations have often been found in the absorptive capacity of the same strain under different conditions. Sometimes this change of condition is definitely due to a physical, chemical, or physiological influence and sometimes, when the reason of the change is unknown, one can only say that apparently spontaneous variations are found in nature.

As an example of physiological influence, artificially introduced, I may refer to the well-known fact that changes are often produced in bacteria by cultivation in immune serum. P. Th. Müller (1903)[1], for example, found that the agglutinability of typhoid bacilli was lowered by growth in immune serum and that this change was accompanied by a diminution in absorptive capacity. But this change, as Paltauf points out, does not always result from the action of immune serum. Some observers have found diminished agglutinability without diminished absorptive capacity; others have found no change in agglutinability; and others again have observed that strains cultivated in immune serum acquire the property of spontaneous agglutination. As another example of physiological influence, it may be mentioned that the characters of an immune serum often depend to an important extent upon the species of animal used for inoculation.

As regards purely physical influences, the effect of heat is the simplest example to take. The very extensive literature on this subject may be briefly summarised by

[1] *München med. Wochenschr.*, p. 13.

saying that exposure of a culture to a temperature above the normal may enhance, impair, or otherwise modify agglutinability, agglutinogenic capacity, and absorptive capacity.

Altmann and Rauth (1910)[1] give a suggestive example of modification in serological properties produced by chemical means. With a particular strain of *B. coli* they produced a serum which, in agglutination and complement deviation tests, responded to the homologous strain alone. This strain, as shown by a month's passage on agar, remained stable and on separating out individual colonies it was found that all were serologically alike, being identical with the original strain. The strain was then treated by passage on carbol-agar, with the result that it lost its agglutinability. A serum was prepared from it and was found to agglutinate the carbol-strain but not the original strain. The properties of the new strain remained constant when the subcultures were made either on ordinary agar or on carbol-agar. Repeat experiments (three with one strain and one with another) produced similar serological modifications by passage on carbol-agar. During passage and before the change had been fully effected, a strain would react both with the ordinary serum and with the "carbol"-serum, and whilst in this transitional condition a strain would produce a serum agglutinating both kinds of culture. Three kinds of colonies could be obtained from such a strain, some reacting like the whole strain and others only with one or other of the two sera. The authors also found that somewhat similar changes could be produced in strains of *B. coli* by prolonged subculture in ordinary broth, the effect being that they lost their agglutinability with the original serum and sometimes became agglutinable with the "carbol"-serum. They suggest that the indol produced in the culture acted in the same way as phenol.

Bacteriological literature is full of examples of what may be termed natural irregularities or spontaneous variations. Perhaps two quotations will suffice for the purpose of illustrating this point.

Rufus Cole (1904)[2] illustrates differences in agglutinability of different strains of typhoid bacilli. He selected five laboratory strains, designated E, H, I, W and C, and ascertained their highest agglutinations with a particular serum. The results were: E = 8000; H = 7000; I = 4500; W = 4500; C = 4000. He then prepared a serum with I, one of the poorer agglutinators, and found: E = 3000; I = 700; C = 500. Then he prepared a serum with C and found it agglutinated E up to 3000 but only reached 2000 with C. Finally he compared the absorptive capacities of good and poor agglutinators on a serum which agglutinated E up to 5000. Four absorbing strains were used and reduced the titre for E as follows: E = 200; H = 500; W = 1000; C = 1000. Thus, as Cole points out, higher agglutinability was associated with greater binding capacity, and relatively poor agglutinability was a consistent feature of some of the strains even when these strains were used for the preparation of the serum. An interesting feature brought out by this short series of experiments is that the differences demonstrated between the good and the poor agglutinators are not such as to afford a basis for "serological grouping."

I have quoted this article because, although it only deals with a small amount of material, it exemplifies very well the experience of other observers on a large number of strains.

[1] *Zeitschr. f. Immunitätsforschung.* Orig. VII. 629.
[2] *Zeitschr. f. Hyg.*, XLVI.

Sobernheim and Seligmann (1910)[1] call attention to biological variations in strains of *B. paratyphosus* (B) and *Gaertner*. They point out that if a laboratory only uses one standard serum for each of these groups of organisms, new strains can generally be accounted for, though with some exceptions. But the results are much more complicated if a large number of strains are tested simultaneously with a large number of sera. Their observations are based on examination of 100 paratyphoid and Gaertner strains and 60 sera.

As regards the Gaertner group, they found that a high titre serum agglutinated only a certain number of strains; others were slightly affected, and many others were left untouched. Comparing the individual results with different sera, further differences came to light; the sera failed to tally either as regards degree of agglutination produced or as regards number of strains agglutinated; hardly any of them affected all their strains. Some strains showed changes in course of time in their agglutinability and agglutinogenic properties; they would pass from good into poor agglutinators or the reverse change would occur, and transitional forms were met with. They had two strains which, for a time, were not affected by any Gaertner or paratyphoid serum and produced a serum which agglutinated themselves alone. But these were *bona fide* Gaertner strains to begin with and subsequently reverted to this type, agglutinating with Gaertner serum up to full titre. Then, when the cultures were plated out and examination was made of separate colonies, agglutinable, inagglutinable, and intermediate colonies were found; and cultures from these exhibited corresponding agglutinogenic differences.

Irregularities and variations were also found in some of the paratyphoid (B) strains. Six strains were typical to begin with but gradually changed in agglutinability. They were then plated out and two kinds of colonies were found, (1) round, translucent colonies which agglutinated like paratyphoid (B), and (2) colonies with granular surface and irregular margin which agglutinated with both paratyphoid and Gaertner serum. Colonies of the second type were plated out four times to confirm their purity, always with the same result; they produced pure paratyphoid (B) serum which had not a trace of influence on Gaertner strains. From another strain, originally an ordinary paratyphoid (B), the daughter cultures were found to be of much lower agglutinogenic power. The serum produced was a pure paratyphoid (B) serum but it only acted on some of the paratyphoid (B) strains and on these, for the most part, not completely; on the other hand, it agglutinated strongly and to high titre not only all the strains which reacted to both paratyphoid and Gaertner sera but also those which had been found to give hardly any reaction with other paratyphoid sera. Finally, from a paratyphoid (B) culture they separated out a strain which was agglutinated by Gaertner but not by paratyphoid sera; but it produced a serum of paratyphoid (B) character, with marked preference for strains in the transitional stage. As the authors remark, the above results show that capacity for binding agglutinin is not necessarily parallel with capacity for producing agglutinin.

The view that certain variations in the combining capacities of antigen and antibody may be attributable to changes in the "chemicophysical phase" of one and the same specific substance leads one to

[1] *Deutsche med. Wochenschr.*, p. 351

consider the possible influence of minute variations in stereo-chemical structure.

A striking feature about the chemistry of bacteria is that these organisms have a remarkably selective action upon sugars and other allied compounds which are closely related to each other and differ only, or mainly, in stereo-chemical configuration. This selective action, perhaps comparable to the selective action of certain alkaloids, such as brucine, upon sugars which differ only in stereo-chemical respects, may indicate that in the molecules of the bacterial protoplasm there are groupings, linked to asymmetric carbon atoms, which act as "receptors" for the corresponding groupings linked to the asymmetric carbon atoms in the sugar molecules.

This direct evidence of the importance of stereo-chemical structure suggests that differences and affinities of a stereo-chemical nature may also play an important part in the constitution of antigen and antibody and in the relationship of the one to the other.

In this connection one must refer back to the views expressed by Emil Fischer. Writing in 1898 on "The Significance of Stereo-chemistry for Physiology[1]" he has developed the theory, which he had fore-shadowed in 1894, that the selective action of enzymes depends on their asymmetric structure. Though the nature of enzymes is not definitely known, because they have not been isolated as chemically pure compounds, "yet their resemblance to proteins is so great and their origin from the latter is so probable that they must undoubtedly be regarded as composed of molecules which are optically active and asymmetrical. This," he continues, "has led to the hypothesis that between enzyme and fermentable substance a similarity of molecular configuration must exist, if a reaction is to follow. To make this idea clearer I have used the metaphor of lock and key." He is far from regarding this hypothesis as an established scientific theory, and admits that it cannot be fully substantiated until enzymes are isolated in a pure state and their con-figuration is investigated; but he regards it as a fruitful hypothesis and has found it helpful in the orientation of chemical research.

Fischer's conception is based on his study of the sugars and allied compounds, which has provided extensive corroboration of the principles of stereo-chemistry founded by Pasteur, Le Bel and van't Hoff. Whether the same conception is capable of useful application to immunity problems is another matter; but it is at least worth considering. From the stereo-chemical standpoint, in so far as it may concern immunity problems, some of the salient facts which have been demonstrated by research on pure compounds of known chemical constitution are:

[1] *Zeitschr. f. physiolog. Chemie*, XXVI. 60.

(1) A pure compound may be produced not only in the optically active forms *d* and *l* but also in forms which are optically inactive. In the case of the latter, inactivation or compensation may take place either externally, *i.e.*, by union of a *d* molecule with an *l* molecule, or internally, *i.e.*, by compensation within the molecule of a *d* group and the corresponding *l* group. A compound inactive by external compensation can be split up into equal numbers of *d* and *l* molecules; but when the compensation is internal, similar dissociation is impossible, as the separation of the *d* and *l* groups would involve the disintegration of the molecule. The four tartaric acids are usually quoted as the classical examples of these facts.

(2) Enzymes often exhibit a selective action upon the *d* and *l* forms of the same optically active compound, fermenting the one but leaving the other unaltered; and their action upon compensated forms will depend upon whether these can be dissociated into active forms.

(3) Pure compounds are found which are almost identical in chemical structure, the only differences being a stereo-chemical difference in the position of groups linked to an asymmetric carbon atom, *e.g.*, the "right-handed" or "left-handed" position of the groups —OH and —H. The best known examples of these facts are found in the sugars of the 6th series.

(4) Enzymes have a selective action on substances differing only in the slight degree mentioned in (3).

There is, I think, reasonable ground for expecting that, when the chemistry of proteins is better known, stereo-chemical conceptions will correct and greatly simplify Ehrlich's very elaborate but highly artificial theory of an indefinite multitude of "side-chains." From the bacteriological side, this line of explanation seems indicated, perhaps most definitely, by the irregularities and the apparently anomalous results such as are often met with in agglutination reactions. Such apparent discrepancies, when irreconcilable with the working hypothesis which the investigator finds suitable to the majority of his data, are sometimes dismissed as negligible. A serum which only agglutinates a few of the strains it was expected to agglutinate is "not useful"; a culture which does not agglutinate with its assumed "standard" serum is "in poor condition"; if in later subculture it behaves as had been expected previously, it has "come up to standard"; if it agglutinates well at first but falls off subsequently, it has "deteriorated"; at all events these little incidents or accidents "don't count."

I admit it would be difficult to account for them by a Jack-in-the-box appearance, disappearance, or neutralisation of fixed chemical groups or side chains constituting the postulated collection of antigens and antibodies; and I think the fact of their occurrence suggests a modification of this conception in favour of the view that, in the inter-action of compounds containing asymmetric carbon atoms, many minor

changes of a stereo-chemical nature will occur (*i.e.* changes not involving elimination or introduction of fixed chemical groups), and that these minor changes may often suffice to produce very striking differences in serological reactions.

Some, at least, of the irregularities which have been demonstrated in the agglutination reactions of particular species of bacteria may perhaps be attributed to stereo-chemical differences of one and the same specific substance rather than to the production of an indefinite variety of different chemical components; and these differences may concern agglutinability and agglutinogenic capacity as well as absorptive capacity. Thus there are the differences due to: (1) *Storage*: when tested with the same serum, a freshly prepared culture emulsion may differ in agglutinability from the same emulsion tested after keeping for some time; (2) *Heat*: a heated culture emulsion may differ in agglutinability and agglutinogenic capacity from the same culture unheated; (3) *Subculture*: earlier and later subcultures of the same strain may differ in agglutinability and in agglutinogenic capacity; (4) *Conditions of growth*: changes may be produced by environment in the animal body or by the nature of the medium used for culture. And the possibility of similar non-specific differences must be considered when comparing one strain with another.

In the last paragraph I have been considering conditions affecting antigens. Possibly analogous differences may exist in the stereo-chemical condition of the specific antibodies to a given organism. Such differences may be due to: (5) *The animal body*: when animals of different species, *e.g.*, the rabbit and the horse, are inoculated with the same strain, it is found that some of the sera are more multivalent than others, and similar differences are sometimes found between sera from animals of the same species; (6) *Condition of culture used for immunising*: the sera produced may vary according to the condition of the culture as regards (1), (2), (3) and (4); (7) *Storage of serum*: a serum may deteriorate on keeping. Again, the non-specific conditions (5), (6) and (7) must be considered when comparing the specificity of different sera.

Furthermore, in the reaction between antigen and antibody, varying stereo-chemical conditions of the two substances may suffice to explain some irregularities in tests for absorption of agglutinin. When the optically active part of a simple organic compound is inactivated in its behaviour towards polarised light by the presence of its geometrical counterpart, this inactivation may be (*a*) either complete or partial and (*b*) either readily annulled (external compensation) or firmly fixed (internal compensation). Similarly, the union of agglutinin with antigen may be (*a*) either complete or partial and (*b*) may be firmly fixed or may lead with greater or less readiness to a stereo-chemical re-arrangement.

This stereo-chemical view of the conditions which play a part in determining the chemico-physical and specifically chemical interaction between antigen and antibody in the first phase of the agglutination reaction leads to a comparison between the interaction of antigen with antibody and the interaction of a ferment with a fermentable substance. In the latter reaction, the feature which is commonly conspicuous is

that union between the two substances and consequent chemical alteration of the one (the fermentable substance) is followed by complete, or almost complete, dissociation, leaving the ferment free to act upon more fermentable substance; thus a small amount of ferment may, so long as the medium remains favourable for the reaction, and until a condition of equilibrium has been established, act upon an indefinitely large amount of fermentable substance. In the case of antigen and antibody conditions are different in one important respect, in that antigen is unable to combine with or modify an indefinitely large quantity of antibody, and the amount of combination effected depends, *ceteris paribus*, on the amount of antigen present. When the absorbing strain is used in sufficient amount, it renders the serum incapable of agglutinating a further supply of the same strain, or of other strains which are identical in every chemico-physical detail, just as a medium upon which an enzyme has exerted its full effect will not be influenced by the introduction of a fresh supply of the same enzyme. It will be noted that in making this comparison I think it advantageous to regard the enzyme as comparable to the antigen rather than to the antibody.

This result, the removal of a certain agglutinating capacity from the serum, must involve a chemical interaction taking place in the first stage of the agglutination reaction, because cultures which are devoid of agglutinability, either naturally or as a result of experimental treatment, often retain their capacity for "binding agglutinin."

Taking chemical considerations first and postponing the question of physical influences, the usual, and generally the most convenient, way of expressing the experimental facts is to say that the culture removes agglutinin from the serum. This may be true, but it does not follow that it is accurate to regard antibody as a mixed collection of assorted goods from which a culture removes a greater or smaller number of articles and leaves the rest as they were before the reaction took place. The interaction, when absorption takes place with excess of culture, should probably be regarded not as a simple subtraction of certain fractional parts of antibody but as involving partly a combination with antibody and partly a modification of the residuum, and resulting in a new phase of equilibrium which allows antigen to persist in the uncombined state when in the presence of the modified antibody which remains.

This modification of antibody occasionally manifests itself in a paradoxical manner, when it is found that a serum absorbed with a particular strain gives a higher titre for some other strain than it did before absorption. Obviously, in this case absorption has not been a process of simple subtraction of agglutinin, and it is

not very helpful to call it a subtraction of an inhibitory influence; it seems rather a change of "chemico-physical phase" in the direction of increased activity; and in other cases, where the titre of the absorbed serum is unaltered for some strains and greatly or slightly diminished for others, the changed state of residual antibody is an experimental fact but the postulate that some fractions of antibody have been removed and others left "just as they were" seems too crude to be likely to be true.

Fischer's "lock and key" metaphor, as employed by him in a strictly stereo-chemical sense, certainly seems helpful in the interpretation of immunity reactions, if applied in the right place. In the first stage of agglutination the union of antigen and antibody may be regarded as determined by their stereo-chemical configuration, like the union of enzyme and fermentable substance, though antigen is incapable of uniting with an indefinitely large quantity of antibody, because union is not followed by simple dissociation (catalytic action). The union appears to be associated with the production of stereo-chemical changes in the residual antibody which render impossible any further union with the particular stereo-chemical type of antigen employed.

At the same time it must not be assumed that this conception will explain everything. There are not only qualitative, or stereo-chemical, but also quantitative differences of absorptive capacity, the latter probably of a colloidal or physical rather than of a chemical nature. Here it is difficult, if not impossible, to draw a sharp distinction between the first and the second stage of the agglutination reaction. When the reaction has been completed, three facts usually stand out prominently. (1) For the production of agglutination some strains require more agglutinin than others, as shown by differences in titre. (2) The amount of agglutinin removed in "saturation" experiments is more than enough to have agglutinated the whole of the culture used, i.e. a much smaller quantity of serum would have been sufficient for agglutination. (3) The quantity of culture required to "absorb" the same amount of agglutinin often differs with different strains.

These facts suggest physical as well as chemical influence. As the reaction is a lengthy process, as compared with the prompt interaction of simple chemical compounds, it may be considered that the "period of impression," as well as "agglutination proper," requires considerable time for its completion, though it may commence immediately, and also that the physical factors characteristic of the second stage begin to exert their influence before the process of chemical union, characteristic of the first stage, has come to an end. In other words, the "period of

impression" may be associated with physical absorption as well as with chemical interaction.

Hence differentiation by absorption is not a purely qualitative criterion in the chemical sense, because the results obtained are partly determined by quantitative or physical conditions. In so far as the latter can be disregarded, the more strictly chemical conditions emerge more clearly, viz.: (1) the presence of antigen and antibody which are *specific*, in that they are of definite chemical constitution, but may exist in one or other of several different geometrical shapes; (2) the *special* stereo-chemical condition of antigen and antibody which determines whether, "like lock and key," they can approach each other closely enough for the production of a chemical reaction.

If it be possible, as suggested in the preceding discussion, to reconcile the fundamental principles of Ehrlich and Bordet by the connecting link of stereo-chemistry, conceptions of classification by serological reactions will need revision.

Owing to the extreme complexity of the chemical substances concerned and the lack of accurate knowledge as to their nature, this is a problem which is very far from solution; but, though direct chemical analysis of antigen is at present impossible, some advance in this direction may be made on the bacteriological side by analysis of the serological effects attributable to those variations in the structure of antigen which are probably of a stereo-chemical nature.

Detailed study of agglutination, absorption of agglutinin, and agglutinogenic capacity has already proved of value by showing the complexity and variability of serological reactions amongst different members of one species, particularly when many strains and many sera are used and compared. Some strains exhibit one phase of this complexity and some another; and so strains may be sorted out into a considerable number of groups (overlapping to greater or less extent) according to the phase which each exhibits. The number of groups will depend on the number of strains investigated and on the range of serological tests employed; it will increase from time to time as the investigation assumes wider dimensions. Meinicke remarked about the grouping of his cholera vibrios by absorption tests that such a classification is of no practical utility. But the demonstration that such serological differences are forthcoming serves two useful purposes; (1) it shows the unity, underlying minor differences, which characterises members of a species; and (2) it shows that the erection of these minor differences into class distinctions, attributable to the presence of distinct and separable antigens,

would lead to such a large and confusing subdivision as to invalidate the hypothesis on which it was based.

As Uhlenhuth and others have remarked, confusion may not arise if attention is limited to a small number of stock laboratory strains and a few sera; some of these strains might absorb homologous agglutinin from one serum and the rest might absorb it from a second serum, and so no more than two groups would need to be postulated. The confusion comes into prominence when a large and unselected series of freshly isolated strains of a species are fully tested in all their serological capacities.

It must also be borne in mind that serological differences brought out by agglutination tests, though of minor importance in some respects, may be associated with differences of an antitoxic or antibacterial nature, and so may possibly give a clue to the selection of sera for therapeutic purposes.

It is generally agreed that for some organisms, such as the cholera vibrio, antibody is of a simpler nature than is the case with many other species of bacteria wherein strains differ from one another in both agglutinability and agglutinogenic capacity. Here again it may be possible to borrow an analogy from organic chemistry. If the simpler antibody be compared to a sugar of the sixth series, say glucose or galactose, the more complex antibody may perhaps be compared to one of the higher sugars, say lactose, which can be split up into simpler sugars (in this case into glucose and galactose). The species with the complex antigen may produce corresponding agglutinins which reveal a strong indication of division into two groups, though closer enquiry may show that these groups are not sharply separable, and that some strains possess characteristics of both groups. That is what might be expected from a "lactose" antigen or antibody which, under varying conditions affecting its stability, might present resemblances sometimes to glucose, sometimes to galactose, and sometimes to both. That might explain why a strain might not be consistently "glucose" (or "galactose") in both agglutinability and agglutinogenic capacity.

As regards differences between agglutinability and absorptive capacity (agglutination with a certain serum but failure to remove agglutinin for the strain producing the serum), the explanation might be referred, as with the less complex cholera vibrio, to stereo-chemical variation.

Practical Significance.

These theoretical questions have a direct bearing on diagnosis by agglutination tests, particularly concerning the application of Castellani's method to problems other than those of mixed infection.

In a mixed infection, *e.g.* one produced by two organisms, A and B, of undoubtedly different species, everyone would concede that two different antigens are concerned, with correspondingly different antibodies, and that Castellani's method of separating them out is often useful, particularly when there happens to be some accidental or at least non-specific interaction between A antigen and B antibody. In such a case there is no urgent need to formulate any particular theory as to the nature of the reaction. Admittedly, antigen is a very complex substance, and so is antibody; the question whether the interaction takes place between A as a whole and B as a whole (with resultant changes in residual A and B) or merely between one separate non-specific component of A and another similar component of B (residual A and B being unaffected) need not be answered, because either alternative would be in accordance with Castellani"s results.

But with two strains known to be of the same species, *e.g.* two cholera strains, wherein absorption tests revealed differences between the two antigens, one's theoretical conception of the interaction between antigen and antibody must be substantiated. Here there is no justification for the assumption that the differences are due to the presence of a non-specific component in the antigen of each strain. Such an assumption would soon be found to be untenable if applied to a large number of strains of this species; so many components of antigen would then turn out to be non-specific that ultimately no demonstrable components of specific antigen would be left.

Still more necessary is it to challenge underlying theoretical assumptions as to non-specific components in dealing with a case presented for diagnosis, *i.e.* in answering the question whether an unknown organism can be proved to be of different species from a known organism by the adoption of the Castellani method. If this question is seriously *sub judice*, one must give the organism in question the benefit of a fair trial, and therefore one must recognise the validity of the plea that the unknown and the known organism may be of the same species though differing, like cholera vibrios, in absorptive capacity.

Hence, in dealing with organisms which may possibly be of the same species, in virtue of their morphological and cultural identity, one cannot accept any deductions from theoretical considerations of immunity which are so framed as to permit an arbitrary sorting out of the agglutinins produced by such organisms into specific and non-specific components.

Observations by Drs Griffith and Scott.

I will now bring my discussion of the absorption test into relation with the observations made by Dr Griffith and Dr Scott.

Dr Griffith has shown that simple agglutination tests suffice to effect a rough division of meningococci into two main groups, provided that carefully selected sera are used; but this division cannot be strictly maintained with all sera, because the grouping produced by some would be different from that produced by others. His explanation is that the grouping, being based on a response to the predominant agglutinins contained in the serum, is determined by the antigen used to produce these; but strains, though alike in agglutinability with the selected "group" sera, may differ from each other in the properties of their antigens and hence may produce different agglutinins. This rough grouping has, however, been found very useful for orientation purposes, before proceeding to a more precise analysis of antigen. In comparing cerebro-spinal with naso-pharyngeal strains, it has brought into prominence the fact that, whereas the two collections of strains are about equally represented in Group II, representatives of Group I are common in the former collection but rare in the latter.

If the system of grouping were infallible, one might infer that Group I antigen is rarely present in the naso-pharyngeal meningococcus of the non-contact. But as the system is far from being perfect, a second alternative has to be considered; the naso-pharyngeal meningococcus may contain Group I antigen and its presence may be demonstrated by the use of other sera prepared from cerebro-spinal strains containing that antigen. Three such sera have been made by Dr Griffith and have been found to agglutinate several naso-pharyngeal strains strongly enough to indicate the presence of Group I antigen in these.

This last observation furnishes a clue to the analysis of antigen which Dr Griffith has followed up in his study of agglutinogenic capacity. Sera were prepared from six naso-pharyngeal strains, none of which could be identified with Group I by simple agglutination tests. The sera, however, gave good agglutination with several cerebro-spinal strains in both groups and exhibited a more uniform influence on those belonging to Group I; they generally failed to agglutinate the naso-pharyngeal strains which were agglutinated by the standard Group II serum, but were fairly consistent in agglutinating those naso-pharyngeal strains which were not affected by this serum. Tests for agglutinogenic capacity, therefore, show that certain naso-pharyngeal strains possess

both Group I and Group II antigens. Similar characters have been demonstrated for a few of the cerebro-spinal strains which could not be classified by simple agglutination as either Group I or Group II.

Dr Griffith's next step in the analysis of antigen was to resort to the absorption test, which, he finds, gives more precise information than simple agglutination as to the combination of agglutinin and antigen and defines more clearly than the agglutinogenic test the degree of relationship between the antigens of different strains. Taking 22 spinal strains of Group I and testing them with six sera, he has found that they differ in range of absorptive capacity. Some strains remove from the sera all the agglutinins demonstrable, which he designates A, B, and C; other strains remove C only, others B and C, and others A and C. These results he has confirmed by increasing the quantities of culture used for absorption; as the differences remained unaffected, he concludes that they are qualitative and not quantitative in character. Corresponding to these differences he postulates three components, A, B, and C, in the antigen of Group I strains. From a similar analysis of Group II spinal strains he has shown that there are at least four different Group II agglutinins.

On comparing naso-pharyngeal and cerebro-spinal strains as regards combining capacity, he finds that those of the former origin which agglutinate well with Group II spinal sera also exhaust the homologous agglutinin of one or more of the four representative sera.

Of the remaining naso-pharyngeal strains, a few, which were agglutinated by Group I sera, have been found to absorb one or more of the three Group I agglutinins.

There is, however, a larger residue of naso-pharyngeal strains which could not be classed by agglutinability as either Group I or Group II. By means of the absorption method, taken in conjunction with agglutinogenic tests, Dr Griffith has shown that several of these possess components of both Group I and Group II antigen. In this respect they resemble two spinal strains which also failed to agglutinate distinctively with Group I and Group II sera and were unable to absorb any of the three components of Group I agglutinin.

The observations which I have briefly summarised in the preceding paragraphs enable Dr Griffith to expand his conception of the nature of meningococcus antigen. He regards it as a substance which all meningococci possess in common, irrespective of their origin and irrespective of their classification as Group I, or Group II, or indeterminate. But it is a complex substance and different strains manifest its com-

plexity in different degrees and in different ways. In some strains, which cannot be grouped either as I or II, he regards antigen as being in its least complex phase, with the Group I and the Group II elements about equally balanced. In others complexity is increased by a preponderance of one, two, or all three of the Group I elements designated A, B and C. In others, again, there is a similar preponderance of one or more of the elements characteristic of Group II. Underlying these differences, however, there is the same specific substance possessed by all strains in common; and it is the presence of this which explains why strains which differ markedly in agglutinability can be shown to be inter-related by agglutinogenic and absorption tests.

In support of this view Dr Griffith calls attention to modifications in antigenic capacity which some of his strains have exhibited in the course of sub-culture. These changes, he considers, can be explained as modifications of a primary antigenic substance in one or other of two directions, involving increase or diminution of complexity. A similar conception, he holds, would explain changes in antigenic characters which may be attributable to the influence. of the human tissues and are characterised in cases of cerebro-spinal fever by the acquired capacity of invading the meninges, a capacity which appears to be much more capable of development in strains possessing the Group I type of antigen than in strains with the less complex antigens which cannot be relegated either to Group I or Group II. These modifications of antigen may, he suggests, run parallel with the curve charting the course of an epidemic, increased complexity of antigen being associated with the upward curve and decreased complexity with the downward curve.

In commenting on Dr Griffith's observations I think it will be useful to call attention to the differences in mental attitude which bacteriologists have adopted towards the absorption test. Some observers, impressed by the fact that this test is often a valuable aid to diagnosis, emphasise, and perhaps over-emphasise, its value as a bacteriological criterion. On the other hand, there is a school of bacteriologists who emphasise, perhaps unduly, the marked differences in absorptive capacity which may be exhibited by strains undoubtedly of the same species; hence they are disposed to minimise the value of the method for diagnostic purposes. I think Dr Griffith's work will help to bring about a reconciliation between these opposing views. He has shown that the absorption method is of great value in throwing light upon the structure of antigen; but, as the structure is complex and liable to variation, great care is requisite in the interpretation of absorption results. On

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the other hand, he does not find that absorptive capacities are so irregular as to be unsuitable for scientific analysis; he shows that their variations appear to be determined by definite principles, and that they are capable of a classification which is systematic though as yet incomplete. He considers that the variations are dependent on minute changes of structure; these, I have suggested, may be largely determined by conditions of a stereo-chemical nature. I agree with his view that the changes can be explained as modifications of a primary antigenic substance; and I think this explanation is preferable to postulating the introduction from without of an antigen originally alien to the strain, or the removal from within of an antigen which the strain originally possessed.

Dr Scott has made an independent investigation of the same problem, using a different set of strains from those employed by Dr Griffith. Like Dr Griffith, he has found that his strains can be roughly divided into two main groups and that the cerebro-spinal strains are well represented in both groups, whilst the naso-pharyngeal strains preponderate in Group II, but are conspicuously rare in Group I. On minute analysis he finds that this rough subdivision does not suffice for a complete classification of the strains he has examined. Simple agglutination reactions alone afforded an indication that, in addition to the two main groups, there were at least five smaller groups more or less related to Group I and at least two small groups related to Group II. This further subdivision he has confirmed and rendered more precise by the application of tests for the absorption of agglutinin. But the adoption of this classification for practical purposes was found to be confronted by two difficulties; variations in agglutinability and absorptive capacity were so great as to make the classification uncertain, and some strains were found, both spinal and pharyngeal, which could not be placed as serological members of any of the groups. Hence Dr Scott concludes that it is impossible to regard his types or groups as representing distinct classes limited by hard-and-fast lines.

I agree with this last conclusion of Dr Scott's. In other respects I regard his results as being confirmatory of Dr Griffith's in their bearing on the diagnostic significance of serological reactions. Where the results of the two observers do not tally exactly, the differences are probably attributable to the use of different sera. For example, Dr Scott does not appear to have obtained any sera from Group I strains presenting the high degree of antigenic complexity exhibited by some of Dr Griffith's Group I strains.

CONCLUSIONS.

In my introduction to this report I raised the question whether serological tests could be found which would differentiate cerebro-spinal meningococci from naso-pharyngeal meningococci carried by non-contacts. This question opens out a wide problem, which I have presented as a series of definite issues, following one after the other, and each demanding a practical solution. Reverting to the order in which I set them out, I think the following answers may be returned to the questions raised as to the value of serological tests for the diagnosis of the meningococcus.

(1) How many standard sera would be required? In replying to this question one must first raise objection to the term "standard." Serologically, the meningococcus is unlike such bacteria as the typhoid bacillus and the cholera vibrio which, on the whole, are uniformly good agglutinators and therefore may be expected to conform to a serological standard. The meningococcus is one of many organisms which are much less constant in their response to agglutination tests, no matter what serum is employed, and therefore do not necessarily conform to any serological standard. The more appropriate question would be: How many sera would be required to form a useful aid to the diagnosis of the meningococcus? The answer is that two sera would suffice for the greater number of strains, provided that the one was a typical Group I and the other a typical Group II serum. There would remain some strains which were not hit off by either serum. If sera were produced from some of these, the number of strains not found amenable to the agglutination test would be diminished, but one cannot say more than that; it would be quite arbitrary and unjustifiable to fix a numerical limit of three, four, or any greater number of sera and to claim that every meningococcus must agglutinate with one or other of these.

(2) Would there be identity of standards in different laboratories? Every laboratory would have a Group I and a Group II serum, but probably the two sera employed in different laboratories would not be identical, as the range of activity of these sera depends to a very important extent on the particular strains used for immunisation. As regards subsidiary sera there would be still less likelihood of identity.

(3) Would simple agglutination tests necessarily be diagnostic? Irrespective of cultural tests, agglutination is not sufficient, because other organisms, *e.g.* the gonococcus, may agglutinate with a meningococcus serum. With organisms corresponding to the meningococcus in

all other laboratory tests, a positive agglutination result is confirmatory, but a negative result, even with several sera, is not decisive.

(4) What is the value of agglutination when supplemented by tests for absorption of agglutinin? A positive result of the absorption test is confirmatory, but absorptive capacities are too irregular to justify any diagnostic significance from negative results.

(5) Is the absorption method valid? The absorption test proves nothing when the result is negative, *i.e.* negative results do not disprove membership of a species, as indicated by other biological characters.

(6) What is the value of sera prepared with naso-pharyngeal strains from non-contacts? When not identical with sera obtained from cerebro-spinal strains, they demonstrate inter-relationship between cerebro-spinal strains and such naso-pharyngeal strains as are not agglutinated by the cerebro-spinal sera available. They show no indications of any serological characters common to naso-pharyngeal strains and distinguishing these from strains of cerebro-spinal origin.

(7) How do theoretical considerations of immunity affect the practical problem of diagnosis? They show that Castellani's principles of differentiation by absorption of agglutinin, taken in conjunction with Durham's postulate of multiple components of antigen and antibody, cannot be regarded as an infallible criterion for the identification of species.

(8) Are serological tests necessary before deciding whether an organism is or is not a meningococcus, *i.e.* capable or incapable, under favourable circumstances, of producing cerebro-spinal fever? No; cultural tests, if adequately performed, will suffice.

APPENDIX.

THE RELATION OF THE BOARD'S BACTERIOLOGICAL INVESTIGATIONS TO OTHER RECENT ENQUIRIES ON MENINGOCOCCUS CARRIERS.

In my last report I presented a historical survey of the literature up to the end of 1914, *i.e.* up to the period immediately preceding the epidemic of cerebro-spinal fever in·this country. This outbreak has led to a large number of investigations throughout the country, dealing chiefly with the meningococcus problem as it has affected the military forces.

The bacteriological reports on this work, for the year 1915, have been reviewed by a Special Advisory Committee which reported to the Medical Research Committee in 1916[1]. The Committee states (p. 32)

[1] *Medical Research Committee. Special Report Series.* No. 2.

that "a very large part of the work of most of the reporters has lain in the routine examination of the pharynx of contacts with cases of cerebro-spinal fever." It continues (p. 33):—"In contrast to their experiences with the cerebro-spinal fluid, the great majority of the reporters complain of the unsatisfactory results of the methods for determining the presence of the meningococcus in the naso-pharynx....The work is tedious and beset with pitfalls, while its results were often found ambiguous. Two of the reporters, indeed, express a doubt whether the swabbing of contacts is of sufficient value to be worth the trouble involved." On the use of agglutination as a test for the meningococcus, the Committee says (p. 14):—"Some of the reporters have tried this mode of diagnosis. The best methods for its employment were determined some years ago, chiefly by the German workers....Most of those who have employed this test seem to have found it capricious and unreliable." And again (p. 33):—"In the present series of reports agglutination has been little used as a confirmatory test, or tried and found too unreliable to be of service. Major Gordon now believes that the methods and special sera which have been introduced at the Millbank laboratories will in future form the most speedy and reliable confirmatory tests available."

Subsequent to the issue of this Committee's report, the work of Gordon and his associates has been brought up to date in a special volume, published in 1917[1]. The relationship of this work to that conducted in the Board's Laboratory calls for some notice.

The laboratory work done for the military authorities was organised with a view to dealing with a special emergency. Cases of cerebro-spinal fever had occurred amongst the troops and, with the object of preventing the spread of the disease, it was decided to swab contacts and isolate all men found to be carriers of cocci which might be regarded as dangerous. The dangerous cocci were eventually defined as those which were found by every available test, serological as well as cultural, to be identical with meningococci isolated from recent cases of cerebro-spinal fever. Of the contacts examined, those found to be carriers of such cocci were to be reported as "positive," the others as "negative." To facilitate prompt diagnosis, a routine procedure of laboratory tests was laid down, and special culture media were provided, together with certain varieties of sera prepared from strains regarded as representative of the different varieties of meningococci discovered in the epidemic then prevailing.

[1] *Medical Research Committee. Special Report Series.* No. 3.

The work in the Board's Laboratory was arranged with a different object in view. Routine work on the meningococcus was limited to the diagnosis of specimens of cerebro-spinal fluid sent by medical officers of health in England and Wales, whilst the carrier question was treated entirely as a research problem, involving enquiry, irrespective of previous bacteriological findings or provisionally accepted opinions, as to the presence or absence of meningococci in the naso-pharynx of non-contacts. Hence there is very little basis for comparison between the results obtained in the Board's Laboratory and the laboratory data furnished to the military authorities.

The investigation of the non-contacts at St Bartholomew's Hospital will serve as an illustration. When Dr Griffith and I first found that a considerable number of these patients yielded strains which were culturally indistinguishable from meningococci, we refrained from making a "positive" diagnosis for six months or more, because we considered that the serological reactions of the strains should be fully worked out before arriving at a decision. Again, when certain of these strains failed to agglutinate with sera prepared from cerebro-spinal strains, we did not regard this result as decisive in favour of a "negative" diagnosis, because sometimes meningococci from cases of cerebro-spinal fever, like pneumococci from cases of lobar pneumonia, may fail to agglutinate with any serum prepared from so-called "standard" strains. In short, it was not our business to follow a prescribed schedule of tests which would determine automatically for each strain whether it was to be reported as "positive" or "negative"; the task was to make full investigation of the individual idiosyncrasies of both cerebro-spinal and naso-pharyngeal strains; and it was only when this work had been in progress for about a year that it was decided that the latter strains must be regarded as true meningococci. This delay caused no inconvenience, because no restrictions of any sort were contemplated for the carriers discovered.

I have no hesitation in saying that, if we had been required to make prompt diagnosis according to the schedule of procedure laid down by the military authorities, the "negative" returns would have been more numerous and would have included many cases yielding strains which eventually proved to be undoubted meningococci.

As regards laboratory details, I have already referred (p. 69) to the care needed in the interpretation of negative results. The remarks of Gordon on the fermentation tests[1] remind me of further questions,

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[1] *Medical Research Committee. Special Report Series.* No. 3, 1917, p. 3.

which are well worth discussing, as to the value of particular tests for
determining that a given coccus is not a meningococcus. The four
sugars which Gordon discusses are glucose, maltose, galactose, and
saccharose. About the value of the last there is no question, as all
bacteriologists are agreed that a coccus which ferments saccharose is,
ipso facto, not a meningococcus. The other three sugars need more
careful consideration.

With galactose, Gordon observes "there has been diversity of ex-
perience," and I agree with him that this is probably due "to alteration
of this somewhat fragile sugar in steaming." This involves a modifica-
tion of the position which Gordon held in 1907[1]. He then maintained
that cultural and fermentation tests were sufficient for differentiating
meningococci from Gram-negative cocci of the normal throat, without
resort to the agglutination test, which he was "quite unable to re-
commend"; and he regarded failure to ferment galactose as excluding
a coccus from the meningococcus group. Now that he has very frankly
changed his views in the light of subsequent research, I agree with him
that it is better to abandon galactose as an exclusion test. Galactose
has been given a trial in the Board's Laboratory but has not been found
particularly useful. To avoid decomposition, it should be sterilised
separately in 10 per cent. solution before it is added to the medium.
It will then be found that the meningococcus consistently fails to fer-
ment it. There may, however, be some advantage in using laevulose
instead of galactose. This sugar, which also requires careful treatment
to avoid decomposition, is not fermented by the meningococcus, but it
forms acid with some strains of *flavus* which fail to attack saccharose.

With both glucose and maltose Lingelsheim found that all his
strains of meningococci agreed in giving a well-marked acid reaction;
and apparently Gordon's experience, as recorded in his earlier work,
was the same. If the experience of other observers were in agreement
on this point, there would be no disadvantage in omitting the use of
maltose, as Gordon has done in his later investigations. But, as I pointed
out in my previous report (pp. 408–12), several bacteriologists have
found that some strains of meningococci attack maltose much more
strongly than glucose; and this fact has been repeatedly confirmed in
the Board's Laboratory. Not infrequently a freshly isolated strain
of cerebro-spinal origin has failed to give any acid reaction in the
glucose tube; a similar result with a naso-pharyngeal strain would

[1] Report to the Local Government Board on the Micrococcus of Epidemic Cerebrospinal
Meningitis and its Identification.

obviously not justify the exclusion of the latter organism from the class of meningococci. So I think the use of maltose should be retained.

The above considerations indicate that the fermentation tests require careful handling, and that caution is needed in the interpretation of their results, especially when freshly isolated strains are under investigation. When the requisite precautions are taken, these tests must still be regarded as a useful part of the series of cultural tests which determine whether an organism is a meningococcus.

In 1907 Gordon was strongly of opinion that cultural tests were sufficient for the diagnosis of meningococci from the naso-pharynx, and that nothing was to be gained by supplementing them with serological reactions. I am inclined to agree with this view, provided that the cultural tests are rightly conducted. Since 1907 a large amount of agglutination work has been put on record; and when one takes a broad view of its results, instead of focussing attention upon some particular hypothesis as to serological grouping, it is seen that serological tests afford no basis for excluding from the class of meningococci an organism which has been properly identified by cultural tests as belonging to this species. This view is expressed even more strongly by the Special Advisory Committee which reported to the Medical Research Committee in 1916[1]. Discussing naso-pharyngeal cocci, they say: "We should regard a meningococcus-like organism which gave *all* the cultural reactions of the meningococcus as certainly capable of producing meningitis."

In the same paragraph the Committee say:

...it appears to us that the meningococcus is shown to be a good enough "species" in the natural history sense, as species go amongst bacteria. That is to say it can be adequately separated from other Gram-negative cocci by the exercise of reasonable care. By serological means it can be divided up, it is true, into certain immunological races or strains, as will be mentioned a little later, but this need not affect its specific entity.

On p. 15, where they discuss the diagnostic value of serological reactions, they remark:

The German observers endeavoured to prove that the strains found in the throats of non-contacts were "pseudo-meningococci," but they were unable to frame any definition of a pseudo-meningococcus which would not include some undoubted spinal strains. No serum has ever been produced which will certainly distinguish between the genuine organism and the so-called pseudo-meningococcus.

[1] *Medical Research Committee. Special Report Series.* No. 2, p. 10.

And they add:

The evidence which has so far accumulated suggests that comprised under the term meningococcus there are a number of races, differing in their immunological reactions, some apparently more virulent than others, but there is so far no justification for asserting any to be destitute of potential pathogenic powers. How sharply defined and stable these races may be we do not at present know.

The above opinions, it appears to me, present a cautious and accurate review of the position established at the beginning of 1916; and their accuracy is confirmed by the results of subsequent work in the Board's Laboratory, as shown in the present series of reports.

To recapitulate, cultural tests are sufficient for the diagnosis of the meningococcus; confirmation by serological tests, which indicate a subdivision of meningococci into different serological races, is not necessary for deciding whether an organism belongs to the meningococcus species.

This position is quite compatible with the view, now held by the majority of investigators who have studied the subject, that the serological characteristics of meningococci are of considerable interest and importance; it is at variance only with the opinion of extremists who maintain that submission to a particular set of serological tests is the necessary criterion for deciding whether an organism is or is not a meningococcus.

From his reports on the recent epidemic, it is evident that Gordon has radically changed the opinions he expressed in 1907 as to the value of serological reactions. He now attaches high importance to these tests, and has used them as a basis for the subdivision of meningococci into "types." His reports for 1915 have been reviewed in the Medical Research Committee's Report, which expresses (pp. 59—60) the following conclusions as to the value of his serological work:

The "types" defined by Major Gordon by means of the agglutinin absorption test were all from the meninges and had caused epidemic cerebro-spinal fever; that is to say, they are the "epidemic races" which were mainly concerned in the outbreak in England in 1914–15. But when Gordon applied his test to pharyngeal strains, he found that only a portion of them were to be included in his types; it is possible that the residue were non-epidemic and less harmful races.

In pursuit of this last remark, the report develops a theory that meningococci may be divisible into "epidemic" and "domestic" strains. It says:

We may conceive this organism [the meningococcus] to be essentially a saprophyte, though with potentialities of parasitism, divided up, as most bacterial species probably are, into a number, perhaps a large number, of races distinguished by their

immunological reactions. At ordinary times, when cerebro-spinal fever is not epidemic, the saprophytic spread of these races is attended only by the development here and there of sporadic cases of declared disease in the most susceptible elements of the population—the posterior basic meningitis of infants. But from time to time, and hitherto very rarely in this country, individual races attain a greater virulence and their saprophytic spread is attended not only by a larger number of cases of meningitis, but by the attack of young adults, who in ordinary circumstances are immune. Such epidemic strains may be introduced into a community and lead to an outbreak of cerebro-spinal fever; there seems some ground for the belief that at least one out of the three principal strains concerned in last year's epidemic was introduced by the Canadian troops. In any given epidemic there will occur a sapro-phytic spread of the epidemic strains side by side with the domestic and relatively harmless strains indigenous to the locality, so that there are carriers of either, indis-tinguishable except by serological means. Major Gordon suggests that only those carriers need be isolated who bear epidemic strains.

This conception of epidemic cerebro-spinal fever, already we believe held by many epidemiologists, must at present be regarded as a working hypothesis only.

In short, the view of the Medical Research Committee's Report is that serological differences may be correlated with differences of viru-lence and may therefore be of importance in distinguishing highly dangerous from less dangerous naso-pharyngeal strains. This suggestion is interesting, and, if serologically "epidemic" strains were rare except amongst direct contacts, isolation based on the segregation of "epidemic" carriers and release of "domestic" carriers might sometimes be feasible and possibly useful. But the fact is that so-called "epidemic" strains are not rare; they are common even in the non-contact population, amongst which they seldom give rise to cerebro-spinal fever. So it is difficult to see that they are really much more dangerous than the "domestics."

This view of the Medical Research Committee appears, however, to differ very considerably from Gordon's. In his later report (1917) Gordon insists that naso-pharyngeal strains which cannot be identified serologically by means of one or other of his four monovalent sera, prepared from his four "types" of cerebro-spinal meningococci, are not to be regarded as meningococci, though they conform to the cultural and fermentation tests for the meningococcus. He terms them "non-meningococci" or "pseudo-meningococci." He thus disagrees with the opinion of the Medical Research Committee that the meningococcus can be identified by cultural tests alone, and ignores their suggestion that strains not conforming to his serological tests are merely less important varieties of that organism, because less directly associated with out-breaks of cerebro-spinal fever in its epidemic form.

Not only does he ignore this very conciliatory suggestion, evidently expressed as an appreciation of his work, but he seems definitely to repudiate it. He firmly takes his stand on the dictum that cocci not responding to his serological tests are not meningococci. By so doing, I think he places himself in serious difficulties. If, whilst rejecting the compromise suggested by the Medical Research Committee, he had taken his stand on the claim that a coccus cannot be authenticated as a meningococcus unless it agglutinates with a serum prepared from a cerebro-spinal strain, his case would have been difficult but at least it would have been arguable. But one cannot argue over a merely personal dictum, which, expressed as a syllogism, would run: all meningococci must agglutinate with a serum prepared from a cerebro-spinal strain; certain naso-pharyngeal cocci do not agglutinate with Gordon's sera prepared from cerebro-spinal strains; therefore they are not meningococci. One can only remark that the conclusion is invalid.

There is one more aspect in which it is interesting to compare the Board's results with those obtained by investigators working for the military authorities. From the beginning of 1915 onwards, the Board's pathologists have consistently found that the percentage of meningococcus carriers even amongst the general (non-contact) population is notably high. Until recently, the Army investigators have not been able to corroborate this, a circumstance which I think is readily explained by the remarks from the Medical Research Committee's Report quoted at the beginning of this appendix. The Committee states that the investigators complained of the unsatisfactory results of the methods employed for detecting the meningococcus in the naso-pharynx, and adds that "the work is tedious and beset with pitfalls, while its results were often found ambiguous." Last year, however, these reasons for dissatisfaction were evidently removed, and the investigators for the Army proceeded to find high percentages of carriers, which are quite in accordance with the previous findings of the Board. The fact that a high carrier rate had been found since the beginning of 1915 suffices to disprove the hypothesis that these later results are explicable by increase in the carrier rate.