A FURTHER STUDY OF THE SEROLOGICAL REACTIONS OF MENINGOCOCCI FROM THE SPINAL FLUID AND THE NASO-PHARYNX, WITH SPECIAL REFERENCE TO THEIR CLASSIFICATION AND TO THE OCCURRENCE OF THE LATTER AMONG NORMAL PERSONS[1].

By W. M. SCOTT, M.D.

CONTENTS.

[1] Reprinted from *Reports to the Local Government Board on Public Health and Medical Subjects*, n.s., No. 114 (1917), by permission of His Majesty's Stationery Office.

INTRODUCTION.

In a previous report[1] I showed that micro-organisms microscopically and culturally indistinguishable from meningococci of pathogenic origin were present in the naso-pharynx of 30 out of 138 persons (22 per cent.) who had had no demonstrable connection with cases of cerebro-spinal fever. Many of these meningococcus-like organisms were also indistinguishable from known pathogenic strains by serological tests, *i.e.* they agglutinated like known pathogenic meningococci in high dilutions of anti-meningococcal sera; others, however, failed to react to a decisive extent, resembling in this certain pathogenic strains which also failed to give decisive results with any of the sera applied.

The serological tests recorded in that report showed, in addition, that great differences exist among cerebro-spinal as well as among naso-pharyngeal meningococci in their behaviour towards any one monovalent agglutinating serum, but evidence was brought forward to show that the majority of the known pathogenic meningococci examined could be put serologically into two main groups, the members of each being closely alike and well distinguished from those of the other group. At the same time it was shown that there existed both cerebro-spinal and naso-pharyngeal strains which could not be satisfactorily identified by serological tests with either of these two main groups.

In continuing this investigation I have obtained additional strains for study, both from the spinal fluid of cases of cerebro-spinal fever and from the naso-pharynx of persons not associated with the disease, and I have endeavoured in particular to determine by further serological tests the biological relationships of the aberrant strains mentioned above to each other and to the apparently well-defined main groups. For this purpose, I have investigated their agglutinogenic and absorptive capacities, as well as their agglutinability: *i.e.* I have injected them into rabbits and tested upon both typical and atypical strains the agglutinating properties of the sera so produced, and I have compared aberrant with typical strains as regards their capacities of absorbing agglutinin from various sera.

The material employed for the purpose of this report consists of 60 strains of meningococci cultivated from spinal fluid, including 26 not dealt with in my previous investigation, and 71 strains obtained from the naso-pharynx, the majority from people not suspected of connection

[1] *Journ. of Hygiene*, xv. p. 464.

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

with cases of the specific disease, but others from direct contacts. Of these, 55 have been isolated since the completion of my former report. The work has been done in the Board's laboratory, and, as before, in consultation with Drs Eastwood and Griffith of the Board's pathological staff, to whom I wish again to express my indebtedness. I have also to thank various regimental medical officers for permission to take swabs from men under their care.

Origin of the Strains investigated.

Of the strains of cerebro-spinal origin 21 were isolated by Dr Arkwright, of the Lister Institute, during the epidemic of 1915; these were partially studied and described in my previous report (*loc. cit.*), and appear in the present description under the same designations A 1, A 2, etc. Twelve strains I owe to the kindness of Professor Andrewes and Dr Canti, of St Bartholomew's Hospital; these were isolated during the latter part of 1916, and with one exception were from infants under seven years. They are designated B 1, B 2, etc.; they are different from the strains from the same source described by Dr Griffith, except B 12, which is his M 77. Finally, 27 strains were isolated by myself, all but four being from specimens of cerebro-spinal fluid kindly sent me during 1915 and 1916 by Dr Foord Caiger, of the South-Western Isolation Hospital. These are designated C.S. 1, 2, etc., those described in the former report bearing the same numbers in this.

Naso-pharyngeal strains.

These are designated N 1, N 2, etc., the numerical order being determined by the alphabetical order of the names of the persons furnishing the strains and by the date on which each batch of swabs was taken. Strains N 1 to N 16 represent the survivors of those isolated in June and July, 1915, from out-patients attending Lambeth Infirmary, and have already been partially described in my former report. The following strains were collected whilst, in my capacity as local Medical Officer of Health, I was cooperating with the military authorities in the control of epidemic disease. Strains N 17 to N 22 were isolated on February 17, 1916, from soldiers in a garrison town in Kent; N 23 to N 27 were obtained on March 2, 1916, from soldiers in huts near a small village on the Kentish coast, while N 43 to N 49 were obtained from another section of the same battalion at this place on May 11, 1916. None of these had had any known connection with cases of cerebro-spinal fever, but

on May 24, 1916, two cases of the disease occurred in this battalion, and strains N 54 to N 71, isolated on May 26, 1916, were from contacts occupying the same huts as the two cases. Strains N 28 to N 30 were isolated on March 23, 1916, from the personnel of a medical inspection room attached to a battalion in camp in a rural parish in Kent, while strains N 31 to N 42 were taken from soldiers of this battalion on March 28, 1916. None of these had had any direct connection with cases of cerebro-spinal fever, but a soldier from the battalion developed the disease on March 20 while absent on leave, and there was also a civilian case, a child, on the same date, in a house in which two men from the same body were billeted. No other cases were reported to me among or in connection with these soldiers afterwards.

Finally strains N 50 to N 52 were obtained from one civilian and two military contacts of two cases which occurred in billets on March 20 and April 5, 1916, in the town of S——, Kent, while strain N 53 was isolated from a soldier, a positive contact who had been pronounced a chronic carrier by the military authorities concerned.

Among my 71 naso-pharyngeal cases the first 49 strains, N 1 to N 49, are thus derived from non-contacts, while 22, N 50 to N 71, are from contacts; 17 are from civilians, nearly all of adult age, and 54 from soldiers; 16 were isolated during June and July, 1915, and the rest during the first half of 1916.

Morphological and Cultural Characters.

In this connection I have no reason to supplement or modify the details contained in my former report regarding the appearance of colonies on the primary plates; as before, a large proportion of the naso-pharyngeal strains fermented glucose more strongly than maltose; with some, indeed, the fermentation of maltose was extremely feeble on first isolation.

Agglutination Tests.

Sera employed.

As before, monovalent sera alone have been employed, all from rabbits. Nineteen strains altogether have been used for their preparation, and the sera produced are designated by the same symbols as the strain injected. Two were prepared by injection of presumed pathogenic meningococci from the naso-pharynx of acute cases of cerebro-spinal fever. Of these P 1 is the strain which produced the serum "Boscombe" referred to in my former report, while P 2 is "Clayton." Nine were

prepared with strains of cerebro-spinal origin, and are known by the corresponding titles, C.S. 8, C.S. 14, C.S. 16, C.S. 20, A 2, A 10, A 13, A 17, A 24. Of these strains C.S. 8 and A 2 were used in producing the sera of the former report named "Smith" and "Chandler."

Eight naso-pharyngeal strains from non-contacts were employed, and the respective sera are entitled N 1, N 2, N 7, N 10, N 13, N 19, N 29, N 48.

With all but one of these 19 strains I obtained sera agglutinating the homologous coccus completely in dilution of 1–1000 or over; in the case of strain N 7, in spite of prolonged immunisation, the titre failed to surpass 1–800. In this strain abnormal toxicity of the culture caused the death of several rabbits during immunisation, so that a satisfactory serum was not produced; this was the case also with several other strains, both naso-pharyngeal and spinal, the sera obtained being of too low titre for use.

Technique of preparation of sera.

Antigens. The bacterial growth was obtained from slopes of Kutscher's medium or glucose-ascitic agar of 24 hours' incubation, and was injected intravenously while still alive and fresh. The dose employed was increased during immunisation from half a slope to two slopes. In the case of the more "toxic" strains on first injection even a loopful of growth, say 10 mg., was fatal within 48 hours. In some cases the bacterial growth from 24 hour egg slopes was used, but the progress of immunisation was unsatisfactory and eventually in all cases Kutscher or glucose-ascitic agar was employed. Some of the sera did not reach the final high titre until as many as 20 doses had been given, spread over six months. I found that a period of rest in the process of immunisation was useful, since the titre which had before been stationary began to rise satisfactorily again on resumption.

The object of this prolonged immunisation was to procure sera of high titre so that a considerable range of dilution might be employed for comparative tests on different strains, and also so that possible "group agglutinins" might be reduced as much as possible in amount relative to the agglutinins more special for the strain.

Technique of agglutination tests.

The macroscopic method was again used throughout, the mixtures of serum and coccal suspension being incubated for 24 hours at 55° C.

Suspensions were made from glucose-ascitic agar cultures of 24 hours' growth sown from 24 hour egg cultures, the growth being weighed moist and suspended in phenol-saline (0·5 per cent. phenol) at the rate of 2 mg. per c.c.; these were then heated for an hour at 65° C. and kept as stock suspensions. They remained agglutinable for many months, the agglutinability in general tending to increase rather than diminish. In addition, freshly-prepared suspensions (unheated) of the same strength were tested from time to time. These suspensions tended fairly rapidly to become inagglutinable, the rate of change varying very much with different strains.

The mixtures were put up in Durham's tubes calibrated in two equal portions, each containing about 0·3 c.c.; the diluted serum was first put in, then the suspension run in with violence so as to ensure mixture. The use of these small tubes effects a considerable economy in the amount of serum necessary for tests, while providing a sufficient column of suspension for estimating changes resulting from agglutination.

Agglutination of meningococci of cerebro-spinal origin.

In Table I, subjoined, are given the highest titres at which complete agglutination resulted in the case of 60 meningococci of spinal origin with monovalent sera produced by nine strains isolated from adult cases of the specific disease during the epidemic season, two being from the naso-pharynx, and seven from the spinal fluid. As will be seen later, the titre varies with different suspensions of the same strain, but the figures here represent the maximum agglutinability of the various strains with each serum, "maximum" because the most sensitive suspensions were used (old stock heated suspensions) and because the highest value attained in any one experiment was selected.

This table demonstrates the relationships to each other of strains of spinal origin as shown by agglutination. In my former report I showed that agglutination tests with two monovalent sera, "Boscombe" and "Clayton," divided the spinal strains sharply into two groups, the first group agglutinating completely at 1–100 to 1–800 with "Boscombe" serum and either not at all or very slightly at 1–100 with "Clayton" serum, while with the second group the behaviour was reversed. With the stronger sera here employed this grouping is still more evident. Taking the first and last columns, headed sera P 1 and P 2 ("Boscombe" and "Clayton"), it will be seen that the first 24 strains agglutinate to the full titre or nearly with the former and, with two exceptions of 1–500, agglutinate only up to 1–100 or less with the latter; on the other hand

Table I. *Agglutination of spinal strains of meningococci with sera produced by known pathogenic strains.*

Serial No.	Strain	P 1 Serum Titre 1–2000	A 17 Serum Titre 1–1800	C.S. 8 Serum Titre 1–2000	A 13 Serum Titre 1–1500	A 10 Serum Titre 1–1500	A 24 Serum Titre 1–1000	C.S. 14 Serum Titre 1–1500	C.S. 16 Serum Titre 1–1500	P 2 Serum Titre 1–2000
1	C.S. 1	2000	1500	1000	100	100	100	100	100	100
2	C.S. 2	2000	2000	1500	100	100	o	100	100	100
3	C.S. 3	2000	1500	1000	100	500	100	o	100	100
4	C.S. 5	2000	2000	1000	100	500	100	o	100	500
5	C.S. 6	2000	1500	1000	100	1000	100	o	100	100
6	C.S. 7	2000	1000	500	o	500	100	o	100	100
7	C.S. 17 (1)	2000	2000	500	500	500	o	o	o	100
8	C.S. 18	2000	2000	1500	100	100	100	o	o	100
9	C.S. 21	1500	1500	1000	100	100	100	o	100	100
10	C.S. 23	2000	1000	1000	100	100	o	o	o	500
11	C.S. 24	2000	1000	1000	100	100	o	o	o	500
12	C.S. 25	2000	1500	1000	500	100	50	o	o	100
13	C.S. 27	2000	2000	1500	500	500	100	o	o	100
14	A 3	2000	1500	500	100	500	100	o	100	100
15	A 4	2000	2000	1500	100	100	100	o	100	500
16	A 6	2000	1500	1000	500	500	50	o	o	100
17	A 9	2000	2000	1000	100	500	100	o	100	100
18	A 11	2000	2000	1000	100	500	100	o	100	100
19	A 12	2000	2000	1000	100	100	100	o	100	100
20	A 14	2000	2000	1000	500	500	100	o	100	100
21	A 15	2000	2000	1000	100	100	100	o	100	100
22	B 2	1500	2000	1000	100	100	o	o	o	o
23	A 18	2000	1500	1000	100	500	100	o	100	100
24	A 17	1500	1800	1000	100	100	o	o	o	100
25	C.S. 8	500	500	2000	100	100	100	o	o	o
26	A 7	500	500	2000	100	100	100	o	o	o
27	C.S. 20	500	1000	2000	100	500	100	100	o	100
28	C.S. 4	500	1500	1500	1500	1000	100	100	100	100
29	A 13	500	500	1000	1500	1000	100	100	100	100
30	A 23	500	100	o	1000	1500	100	100	500	1000
31	A 1	500	1500	1000	500	500	100	100	500	100
32	A 16	100	500	1000	100	500	100	o	o	o
33	A 2	100	500	500	o	500	500	100	500	100
34	B 12	100	500	500	100	500	o	o	o	o
35	A 10	500	1000	1000	500	1500	50	100	500	100
36	A 24	o	o	100	100	500	1000	1000	1500	2000
37	A 22	o	o	o	o	100	1500	1000	1500	1500
38	C.S. 9	o	100	o	o	o	500	1000	1500	2000
39	C.S. 10	o	100	o	o	o	100	1000	1500	1500
40	C.S. 11	o	100	o	o	100	500	1000	1500	2000
41	C.S. 12	o	o	o	o	100	100	1000	1200	2000
42	C.S. 14	o	100	o	o	500	100	1500	1500	1500
43	C.S. 15	o	100	o	o	100	100	1000	1500	1500
44	C.S. 16	o	o	o	100	500	100	1000	1500	1500
45	C.S. 17 (2)	100	o	o	100	o	100	1000	1200	1500
46	C.S. 19	o	o	o	o	o	100	500	500	2000
47	C.S. 22	o	100	o	o	o	500	1000	1500	2000
48	C.S. 26	o	o	100	o	o	–	1000	1500	2000
49	A 20	o	o	o	o	o	100	1000	1500	1500
50	B 7	o	o	o	o	100	100	1500	1500	1500
51	B 6	o	o	o	o	100	100	1000	1200	1500
52	B 9	o	o	o	o	o	o	1000	1000	1500
53	B 11	o	100	o	o	o	50	500	1000	1500
54	B 4	o	o	o	o	100	100	1000	800	1000
55	A 25	o	100	o	o	100	100	1000	500	1500
56	B 8	o	100	o	o	100	50	500	100	500
57	B 1	o	o	o	o	100	o	500	100	500
58	B 3	o	100	o	o	100	o	100	100	500
59	B 5	o	o	o	o	100	o	100	o	100
60	B 10	o	100	o	o	100	100	o	100	o

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

20 other strains, 36 to 55, agglutinate up to the full titre or nearly with serum P 2 and slightly or not at all with serum P 1. Thus in the case of 44 strains a sharp division into groups exists on the strength of agglutination.

This leaves 16 in which agglutination by these group sera left the classification in doubt. Eleven of these, numbers 25–35, show agglutination with the main Group I serum varying from 100 to 500 and with the main Group II serum from 0 to 100, with one exception which agglutinates to 1000 with the latter serum.

The last-mentioned strain might be classed as Group II, but in the case of all the others the agglutination results leave one either entirely in doubt or with a slight leaning to place them in Group I. Finally five strains, numbers 56–60, do not agglutinate at all with Group I serum and with Group II serum agglutinate at most up to 1–500. The tendency would be to place these in Group II, but again the position must remain in doubt since agglutination to 1–500 with such strong sera cannot be regarded as of decisive significance.

On looking now at the columns under A 17 serum and C.S. 16 serum exactly the same results are obtained except that with A 17 there are greater variations in the high titres obtained with the strains already placed in the main Group I, *e.g.* three strains reach only 1–1000, while nine reach 1–1500, and twelve attain the full titre of 1–2000; further, some of the strains which leaned towards Group I now show more definite alliance, reaching dilutions of 1–1000 in two cases and 1–1500 in other two; the strains 56–60 which inclined to Group II are now almost entirely indifferent, reaching titres of 1–100 at most, a titre which is also attained by the Group I strains with the Group II serum.

Sera A 17 and C.S. 16 thus do not alter the subdivision already noted, and the strains producing them must be regarded as examples of Group I and Group II respectively.

Serum C.S. 14 is closely comparable with C.S. 16 in its effects and picks out again the Group II strains, agglutinating them to approximately full titre.

The results of agglutination with the other sera, C.S. 8, A 13, A 10 and A 24, may be summed up as follows: the first three, prepared with strains already placed as inclining towards Group I, agglutinate Group I strains better than Group II and pick out in each case a small number of the former strains by agglutinating them to the full titre of the serum, while serum A 24, prepared with a strain apparently typical of Group II, agglutinates both groups indifferently, but neither well;

it picks out, however, one other Group II strain besides itself as reacting to the full titre. These two strains evidently form a sub-group of Group II although their agglutination reactions with the other Group II sera give no evidence of their highly individual character as shown by their behaviour towards serum A 24.

The agglutination tests leave unidentified eight strains which react feebly with all the sera, but one of these, strain A 2 (number 33) has been used to produce a serum, the action of which will be described in a later part of this report. In general it agglutinates Group I strains better than Group II, thus confirming its position as near Group I, but it agglutinates none of the spinal strains, except itself, to high titre, and may therefore represent an aberrant strain or may belong to a natural group of which chance has determined that no other representative should fall into my hands.

If one makes a general survey of Table I it may be noted that, if it be read from the top left-hand corner to the bottom right-hand corner, lines drawn from the top of column C.S. 8 to line 36 in column P 2 and from line 25 in column P 1 to the foot of column C.S. 14 include practically all the high agglutination titres between them.

The explanation of this is the fact that the middle of the horizontal line on which the sera are placed is occupied by sera surmised to represent strains intermediate between Group I and Group II, whilst the sera typical of these two groups occupy respectively the left- and right-hand ends; the middle of the vertical line on which are distributed the various strains is occupied by strains similarly surmised to be intermediate. The striking arrangement of the high titres which results in Table I testifies to the correctness of the surmise, and indicates that the agglutinogenic action of these intermediate strains is in agreement with their reactions to the main group sera.

In the case of eight strains out of the 60, classification with the sera employed has failed owing to their poor response to all. Three of these strains appear to incline more to Group I than to Group II, while with the remaining five the inclination is reversed. Each of these sets may represent a true sub-group, or they may consist of highly specialised individuals.

This question could only be answered by preparing sera with each, and testing the agglutinating properties of these sera against a representative selection of strains, *i.e.* by determining their general and special agglutinogenic action.

Variations in agglutinability of the meningococcus in culture.

Special attention to this point was evidently necessary, since neglect in taking account of it might lead to serious error not only in the more purely scientific question of making a classification but in the very practical one of estimating the value of particular anti-meningococcus sera as remedial agents in infections with particular strains of the meningococcus.

As has been stated, heated suspensions of the various strains remain very stable in their agglutinability, the only change, if any, being a gradual one towards increase of sensitiveness, not only towards the sera agglutinating originally to a high titre but also to those agglutinating originally only to a slight extent. In other words, heating the suspension fixes permanently the agglutinating properties which the cocci happen to possess in the fresh unheated state either as a result of the strain's true position among meningococci or as the result of a temporary variation. But such temporary variations between different cultures of the same strain occur to such an extent that suspensions made at different time from subcultures on Kutscher's medium or ascitic agar of strains preserved on egg at 37° C. show very wide divergence.

A good deal of time was spent on trying to correlate these divergences with the differences seen in colonies of the same strain on the same plate. These differences are particularly striking when material taken from an old egg culture (three weeks old or more) is plated out on serum or ascites media. On such plates colonies of two types appear in varying proportion; one type is translucent or very finely granular, resembling in appearance and structure the typical colony as isolated directly from the naso-pharynx or the spinal fluid; the other is opaque, coarsely granular and easily distinguishable. Some colonies are apparently of mixed type and show sharply defined sectors of translucent growth in the opaque disc. After one or two subcultures on serum media these coarsely granular colonies yield only the translucent type of growth, but cultures on egg from single colonies, whether translucent or granular, show after three weeks or so the same mixture on replating.

On investigating the serological reactions of these different types of growth it was found that in some cases the opaque type consisted of relatively inagglutinable cocci, while the translucent type gave the reactions regarded as normal for the strain; but in other cases the behaviour was reversed, and the conclusion I came to was that no correla-

tion between type of growth and agglutinability existed. The same holds true for differences in the size of colonies on the same plate.

The cause for the appearance of the two types of colony may be that in an old egg culture certain cocci have become so altered in the conditions of their growth by existence on an exhausted medium that when given an opportunity of forming colonies on fresh material they are unable at first to grow in the manner typical of meningococci from young and vigorous cultures.

In any case, their rapid reversion to type in this matter indicates that no profound mutation has occurred.

But these observations are of significance in showing that the varying agglutinability of different suspensions of the same strain may be easily explained by the predominance in them either of highly agglutinable or of feebly agglutinable cocci, according as the culture used for the suspension starts from cocci producing readily agglutinable colonies or from those producing the reverse.

In Table II examples are given of the different titres shown by the same strain with different fresh suspensions; all these suspensions were of the standard strength, 2 mg. per c.c. In the case of columns (3) and (4) they were made by subculturing on ascitic-agar single colonies of the different appearances described; column (3) shows the results obtained with the translucent variety and column (4) those with the opaque. Columns (1) and (2) represent growth from colonies of typical appearance from young cultures. The Arabic numerals indicate the dilution of serum at which complete agglutination occurred, while the symbols +, + +, and + + + indicate degrees of agglutination short of complete at 1–100.

It will be observed that at different times with both groups of strains the titre may vary from 1–100 to 1–1,500, *e.g.* with strains A 3 and C.S. 12, and that although in general the variation does not reverse the placing of the various strains in the two main groups, yet in many instances, *e.g.* A 3, A 4, A 6, A 17, C.S. 4, etc., the difference in titre shown, when minimum and maximum agglutinations with the respective group sera are compared, is not such as to give a definite answer as to serological grouping. Hence chance may readily determine that a given strain, though definitely of the main Group I as seen by more extensive tests, may appear as the result of a single test either quite doubtful or even tending towards the wrong group.

The variation just illustrated may, and probably does, depend on differences in the different suspensions of an extrinsic character, *i.e.* in

Table II.

Variations in agglutination with fresh suspensions.

Strain	Serum P 1				Serum P 2			
	1	2	3	4	1	2	3	4
C.S. 1	1500	500	1500	—	+ +	+ + +	100	–
C.S. 2	1500	1000	1500	1500	+ + +	100	100	100
C.S. 3	1500	1500	1500	—	100	100	100	–
C.S. 5	1000	1500	1500	–	+ +	100	500	–
C.S. 6	1000	500	500	1500	o	+ + +	+ +	100
C.S. 7	1500	500	500	1000	+ + +	+ + +	+ + +	100
C.S. 17 (1)	1500	1000	500	1500	100	+ + +	+ +	100
C.S. 18	500	1000	1500	–	+	+ + +	100	–
C.S. 23	1500	500	1500	1500	+	+ +	100	100
C.S. 24	500	1500	1500	–	+ +	+ + +	100	–
A 3	1500	100	1500	1500	+ + +	+ + +	+ +	+ + +
A 4	1500	1000	500	–	500	+ +	+ +	–
A 6	500	500	100	500	+ + +	+ + +	+ + +	+ + +
A 9	1500	1500	1500	–	+ + +	+ + +	+ +	–
A 11	1500	100	1500	–	+ + +	+ + +	+ +	–
A 12	1500	500	–	–	100	+	–	–
A 14	1000	1500	–	–	+ + +	100	–	–
A 15	1000	1500	1000	–	+ +	100	+ +	–
A 18	1500	1500	1500	–	100	+ + +	+ +	–
A 17	+ + +	500	–	–	+	+ + +	–	–
C.S. 8	100	100	o	100	+	+	+ +	o
A 7	1000	100	–	–	+ + +	+	–	–
C.S. 20	500	100	500	–	+ +	+ +	+ + +	–
C.S. 4	o	100	100	100	100	o	+ + +	100
A 13	100	100	100	100	100	+ +	+ + +	100
A 23	o	o	–	–	500	+ +	–	–
A 1	1000	100	o	–	100	100	o	–
A 16	100	100	+	–	+	+ +	+ +	–
A 2	+ +	100	+ + + +	+ +	100	100	100	100
A 10	100	100	–	–	100	100	–	–
A 24	o	o	o	o	1500	100	100	500
A 22	o	o	–	–	1500	1000	–	–
C.S. 9	o	o	o	+	1000	100	1500	1500
C.S. 10	o	o	o	–	1000	100	1000	–
C.S. 11	o	o	o	–	1000	500	1500	–
C.S. 12	o	o	o	+	1500	100	1500	500
C.S. 14	o	o	o	o	1000	100	1000	1000
C.S. 16	o	o	o	–	1000	1000	1000	–
C.S. 19	o	o	o	–	1000	1500	1500	–
C.S. 22	o	o	o	o	1500	1000	100	1500
A 20	o	o	o	o	1500	1000	500	500
A 25	o	+ +	o	o	1000	100	1000	1500

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

the physical condition of the cocci composing them, and not on intrinsic differences of chemical composition such as differentiate the groups. But one strain in my possession, strain C.S. 17, has shown differences of the latter type. Originally on isolation it agglutinated to the full titre with the main Group I serum, and only faintly with that of Group II; when a fresh emulsion was tested a month later, the behaviour was exactly reversed; two months later it again became a typical Group I strain, giving only traces of agglutination with Group II serum. As a result, I have now in my possession two strains isolated from the same colony on a plate inoculated with cerebro-spinal fluid; one strain C.S. 17 (1) behaves in all respects like the 24 strains making up the main Group I, while the other, C.S. 17 (2), is almost equally characteristic of Group II.

The significance of this apparently profound change will be discussed later with special reference to the possibility of original mixture of the two strains.

It may safely be assumed, I think, that if differences of the degrees described occur in strains during culture on artificial media, they are still more likely to occur under the changing conditions of the susceptible or resistant human body.

Further examples of these serological changes will be given when absorption tests have been discussed, since, in estimating the degree of importance to be attached to alteration in serological reactions, agglutination results are not sufficiently decisive; the absorption of agglutinin from a particular serum, it is generally held, is the most satisfactory criterion for establishing identity of an unknown strain with the strain producing the serum.

Classification by agglutination.

I have tried to show in Table I that by agglutination reactions alone it is possible to divide this collection of 60 spinal strains into two main groups supplemented by at least five sub-groups more or less nearly allied to Group I, one sub-group actually within Group II and one probably related to it. The term sub-group in each case indicates merely that the strains representing it are numerically less important than those of the main groups; it does not indicate that the sub-groups lie within the main Groups I and II; they may possess equally pronounced individuality, and all that can be said is that sub-groups 1 to 5 are more nearly allied to Group I while sub-group 7 inclines to Group II. In Table III the reasons are set out for separating each of these sub-groups, as also the numbers of the strains which go to make them up.

TABLE III.

Analysis of Table I showing expanded classification of spinal strains in relation to the two main groups.

No. of Group or Sub-group	Strains composing these: Nos.	Relationships to the main groups		Characteristics peculiar to each set of strains classed as identical
		Group I	Group II	
Main Group I	1–24	These strains constitute this group	Slight and indecisive agglutination with Group II sera	Agglutination to approximately full titre with Group I sera
Sub-group (1)	25 26, 27 (C.S. 8, A 7, C.S. 20)	Agglutinated to 1–500 with main Group I sera	Agglutinated to 1–100 only with Group II sera	Agglutination to full titre with serum C.S. 8, prepared with strain No. 25
Sub-group (2)	28, 29 (A 13. C.S. 4)	Agglutinated to 1–500 with main Group I sera	Agglutinated to 1–100 only with Group II sera	Agglutination to full titre with serum A 13, prepared with strain No. 29
Sub-group (3)	30, 35 (A 23, A 10)	Agglutinated to 1–500 with main Group I sera	Agglutinated to 1–500 with Group II sera	Agglutination to full titre with serum A 10, prepared with strain No. 35
Sub-group (4)	33 (A 2)	Agglutinated to 1–500 with main Group I sera	Agglutinated to 1–500 with Group II sera	Agglutination to full titre with serum A 2, prepared with strain No. 33: no other spinal strain so agglutinated
Sub-group (5)	31, 32, 34 (A 1, A 16, B 12)	Agglutinated to 1–500 or higher but not to full titre with main Group I sera	Agglutinated below 1–500 with Group II sera	Fail to agglutinate to full titre with any spinal sera: ? identical with each other: ? separate individual groups
Main Group II	36 to 55	Agglutinated to slight and indecisive extent by main Group I sera	These strains constitute this group	Agglutination to approximately full titre with Group II sera
Sub-group (6) within Main Group II	36. 37 (A 24, A 22)	As above	Apparently indistinguishable	Agglutination to full titre with serum A 24 prepared with strain No. 36
Sub-group (7)	56–60 (B 8, B 1, B 3, B 6, B 10)	As above	Agglutinated to 1–500 or less with Group II sera	Fail to agglutinate to full titre with any spinal sera; agglutinate better with Group II than with Group I: ? identical with each other: ? separate individual groups

But in practice such classification by agglutination alone is out of the question. It would involve repeated testing of each strain to be classified; the tests would have to be performed with several sera, each known to be different, and strains of established classification would have to be tested at the same time for comparison and as controls.

A single test with selected sera would be insufficient because the agglutination titre of a particular strain for a particular serum may vary within wide limits with suspensions prepared at different times: one suspension may reach even less than half the titre attained by another, and a single indecisive result with a particular serum cannot be regarded as excluding membership of the corresponding group. Furthermore, with certain sub-group sera, *e.g.* serum C.S. 8, the agglutination reactions of some strains foreign to the sub-group differ on the average only slightly from those actually belonging to it, the differences being well within the limit of possible variations.

It will be seen, however, in the following section that by the test for absorption of agglutinin the classification suggested by the agglutination reactions of Table I and defined in Table III receives a considerable amount of support, the differences brought out by absorption being much sharper and less subject to variation.

Absorption Tests.

Technique. My general plan of procedure has been to suspend a weighed quantity of the bacterial growth (from cultures on ascitic agar of 24 hours at 37° C.) in a given amount of serum diluted 1–50. The quantity chosen was that which, with the strain homologous to the particular serum, was necessary to reduce the agglutinating power for itself to nearly *nil*—*i.e.* till the serum, which before absorption agglutinated its homologue, say, to 1–1500, failed to give complete agglutination at 1–100 (though preferably giving distinct traces of agglutination at this dilution). This quantity varied with different sera from 5 mg. to 20 mg. of culture per c.c. of serum diluted 1–50. The mixture was kept in the ice-chest overnight or longer as convenient, a control specimen of the same serum dilution being similarly treated. The cocci were removed by prolonged centrifuging, and the clear fluid was then tested for persisting agglutinin on a suspension of the strain producing the serum; usually other strains more or less closely allied to this were used as test suspensions at the same time.

In a few cases stored suspensions of cocci were employed for absorption instead of the fresh growth, but they were rather unsatisfactory owing to the difficulty of removing them with the centrifuge in cases where agglutination in the mixture was incomplete. The fresh suspensions, on the other hand, even when not agglutinated, were readily removed by the high-speed centrifuge, giving clear supernatant fluid with which to test agglutination.

Agglutination was tested with this fluid undiluted and with increasing dilutions of it up to the maximum known to give complete agglutination in the case of the control specimen, so that degrees of absorption were detectable from "complete," where only traces of agglutinin remained, to "nil," where the serum before and after absorption gave in each case complete agglutination at the highest dilution.

Groups and Sub-Groups.

The main Group I.

In Table IV, subjoined, are given the degrees of absorption from the main Group I serum, P 1, with various spinal strains representing this main group (*vide* Table I), and also with strains more or less closely allied to this group (*vide* Table III); as controls two strains were taken, which on agglutination belonged to Group II. The quantity of culture employed for absorption was in each case 8 mg. suspended in 1 c.c. of P 1 serum diluted 1–50. The suspensions used for testing the persistence or removal of agglutinin were old stock suspensions of high agglutinability.

The symbol C, when used to indicate agglutination, means that complete deposit of the suspended cocci was found, the supernatant fluid being free from turbidity; the symbols + + +, + +, and + indicate diminishing amounts of deposit, the supernatant fluid remaining more or less turbid.

In the columns headed absorption the symbol C indicates that absorption was regarded as *complete*, complete absorption being taken to have occurred when even the 1–100 dilution failed to agglutinate the test suspension completely; absorption designated as + + + means that, though agglutination was still complete at 1–100, it was definitely incomplete at 1–600; while + indicates that agglutination, though complete at 1–600, was incomplete at 1–1000.

It will be seen that the first 24 strains, P 1 to B 2, which (*vide* Tables I and III) were put in the main Group I in virtue of their high agglutination with serum P 1, give complete, or almost complete, absorption of the agglutinin acting on strain P 1, while those strains which showed relationship but not identity with Group I—and also the control Group II strains—remove either small amounts or none. Similarly, the agglutinin present in P 1 serum for another member of the main Group I is in general removed completely by the main Group I strains and barely affected by the others.

TABLE IV.

Absorption of agglutinin from serum P 1.

Serum	Agglutination with Homologous Strain P 1					Agglutination with Related Strain C.S. 3					Absorption of Agglutinin for Homologous Strain P 1	Absorption of Agglutinin for Strain C.S. 3
Control unabsorbed	100 c	600 c	1000 c	1400 c	2000 + + +	100 c	600 c	1000 c	1400 c	2000 + + +		
Absorbed by Strain												
P 1	+	o	o	o	o	+	o	o	o	o	c	c
C.S. 1	+	o	o	o	o	+	o	o	o	o	c	c
C.S. 2	+	o	o	o	o	+	o	o	o	o	c	c
C.S. 3	+ + +	trace	o	o	o	+	o	o	o	o	c	c
C.S. 5	c	+ +	o	o	o	+ +	o	o	o	o	+ + +	c
C.S. 6	c	+ +	o	o	o	+ +	o	o	o	o	+ + +	c
C.S. 7	+ +	o	o	o	o	+	o	o	o	o	c	c
C.S. 17 (1)	c	+ +	o	o	o	+	o	o	o	o	+ + +	c
C.S. 18	+ +	o	o	o	o	+ +	o	o	o	o	c	c
C.S. 21	c	+ +	o	o	o	+ +	o	o	o	o	+ + +	c
C.S. 24	+	o	o	o	o	o	o	o	o	o	c	c
C.S. 25	+	o	o	o	o	o	o	o	o	o	c	c
C.S. 27	+	o	o	o	o	o	o	o	o	o	c	c
A 3	c	o	o	o	o	o	o	o	o	o	+ + +	c
A 4	+ +	o	o	o	o	o	o	o	o	o	c	c
A 6	c	o	o	o	o	+ +	o	o	o	o	+ + +	c
A 9	+ + +	o	o	o	o	+	o	o	o	o	c	c
A 11	+	o	o	o	o	o	o	o	o	o	c	c
A 12	+	o	o	o	o	o	o	o	o	o	c	c
A 14	c	o	o	o	o	+ +	o	o	o	o	+ + +	c
A 15	c	+ +	o	o	o	c	+	o	o	o	+ + +	+ + +
A 18	c	o	o	o	o	o	o	o	o	o	+ + +	c
A 17	c	+	o	o	o	c	+ +	o	o	o	+ + +	+ + +
B 2	c	o	o	o	o	+ +	o	o	o	o	+ + +	c
C.S. 8	c	c	+ + +	+ +	+	c	c	c	c	+ +	+	trace?
A 7	c	c	c	c	+ +	c	c	c	c	+ +	trace?	trace?
C.S. 20	c	c	c	c	+ +	c	c	c	c	+ +	trace?	trace?
C.S. 4	c	c	+ + +	+ +	+	c	c	c	c	+ +	+	trace?
A 13	c	c	+ + +	+ +	+	c	c	c	c	+	+	trace?
A 1	c	c	+ + +	+	o	c	c	c	c	+	+	trace?
A 2	c	c	c	+ +	o	c	c	c	c	+	trace	trace?
A 16	c	c	+ +	+	o	c	c	c	+ + +	+ +	+	trace
B 12	c	c	c	c	+	c	c	c	c	+	trace?	trace?
B 3	c	c	c	c	+	c	c	c	c	+	trace?	trace?
A 10	c	c	c	c	+ + +	c	c	c	c	+ +	o	trace?
C.S. 14	c	c	c	c	+ + +	c	c	c	c	+ +	o	trace?
*N 19	+ +	o	o	o	o	+	o	o	o	o	c	c

* To avoid repetition of the table the absorptive capacity of N 19 is inserted here and will be discussed on p. 231 in its place as a naso-pharyngeal strain.

This result, which has been many times repeated, is very clear and definite; the minor variations in absorptive capacity, indicated in this table among the different strains within the main group, have not been reproduced with regularity in duplicate experiments, and probably indicate only slight temporary alterations in individual culture masses of the various strains. This main Group I is thus well defined and relatively homogeneous; and I may note incidentally that similar behaviour in the case of certain coli-form bacilli of the "food-poisoning" group has been used by some authorities as the criterion for establishing biological species.

In the following table (Table V) are indicated the results of absorption from another main Group I serum, the serum produced by strain A 17, which, though irregular in its agglutination with serum P 1 (*vide* Table II), yet at times reaches the full titre, and, as has just been seen, gives almost complete absorption of agglutinin from it.

TABLE V.

Absorption of agglutinin from serum A 17.

Serum	Agglutination with Homologous Strain A 17				Agglutination with Related Strain C.S. 2				Absorption of Agglutinin for Homologous Strain A 17	Absorption of Agglutinin for Strain C.S. 2
	100	500	1000	1500	100	500	1000	1500		
Control unabsorbed	c	c	c	c	c	c	c	c		
Absorbed by Strain										
C.S. 1 . .	c	trace	o	o	+ + +	o	o	o	+ + +	c
C.S. 3 . .	c	trace	o	o	+ + +	o	o	o	+ + +	c
C.S. 5 . .	+ + +	o	o	o	+ + +	o	o	o	c	c
C.S. 7 . .	c	+	o	o	+ + +	o	o	o	+ + +	c
C.S. 17 (1) .	c	trace	o	o	+ + +	o	o	o	+ + +	c
C.S. 21 .	+ + +	o	o	o	+	o	o	o	c	c
C.S. 27 .	c	o	o	o	+ + +	o	o	o	+ + +	c
A 3 . .	+ + +	o	o	o	+ + +	o	o	o	c	c
A 6 . .	c	+ +	o	o	c	o	o	o	+ + +	+ + +
A 9 . .	c	+ +	o	o	c	o	o	o	+ + +	+ + +
A 15 . .	c	+	o	o	c	o	o	o	+ + +	+ + +
A 17 . .	+ + +	+	o	o	c	o	o	o	c	+ + +
B 2 . .	c	o	o	o	+ + +	o	o	o	+ + +	c
A 7 . .	c	c	c	c	c	c	c	+ + +	o	trace
C.S. 4 . .	c	c	c	c	c	c	c	c	o	o
A 1 . .	c	c	c	c	c	c	c	+	o	trace
B 12 . .	c	c	c	c	c	c	c	+ + +	o	trace
*N 19 . .	+ + +	o	o	o	+	o	o	o	c	c

* Naso-pharyngeal strain, see p. 231.

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

The quantities used were 10 mg. of culture per c.c. of 1–50 dilution of the serum and the test emulsions were the homologous strain A 17 and another main Group I strain, C.S. 2, of typical behaviour.

The results are very similar to those shown in Table IV. Differences in absorptive capacity between C and + + + coincide in some cases with similar differences in the former table, while in other cases absorptions indicated as + + + in Table IV here reach completeness. Such variations and coincidences may represent a tendency to sub-grouping within this main group, but, as has been noted above, they are not sufficiently regular to justify any such conclusion.

The object of inserting Table V is to show again the homogeneous nature of the main Group I, seen in Table IV; for this purpose the serum produced by A 17 was selected, as A 17 is one of the main group strains which showed the greatest tendency to diverge; further, A 17 is a strain of spinal origin, whereas P 1, used to produce the serum P 1, which has been chosen as the main Group I serum, was isolated from the naso-pharynx; yet the results with A 17 serum are practically indistinguishable.

Sub-Group (1).

In the next table (Table VI) absorption tests are given, using the same strains as in Table IV, but absorbing from the serum C.S. 8, produced by one of the strains which, on agglutination results, show relationship but certainly not identity with the main Group I. The technical details are exactly the same, and the masses of culture employed for absorption came from the same ascitic agar slopes as in the experiment summarised in Table IV; the symbols employed are also the same.

The results are equally sharp and definite. Absorption is complete with the three strains (including the homologue) which in Table I were seen to reach the full titre with this serum, namely, strains C.S. 8, A 7 and C.S. 20. On the other hand, the numerous strains of the main Group I, which agglutinated fairly strongly with serum C.S. 8, fail entirely, or almost entirely, to remove the agglutinin for either strain C.S. 8 or A 7, as also do the other strains which were shown to be related, but not identical with the main Group I.

Here, therefore, there is a group, which may be called the C.S. 8 group (sub-group (1) in Table III), quite as well defined apparently as the main Group I, the three strains composing it having on the results of absorption as much right to specific differentiation as the much larger group, although the agglutinogenic action of the type strain C.S. 8,

Table VI.

Absorption of agglutinin from serum C.S. 8.

Serum	Agglutination with Homologous Strain C.S. 8					Agglutination with Related Strain A 7					Absorption of Agglutinin for Homologous Strain C.S. 8	Absorption of Agglutinin for Strain A 7
Control unabsorbed	100	600	1000	1400	2000	100	600	1000	1400	2000		
Absorbed by Strain	c	c	c	c	++	c	c	c	c	+++		
P 1 . .	c	c	+++	+	o	c	c	c	++	o	+	trace
C.S. 1 . .	c	c	c	++	o	c	c	c	+++	o	trace	trace
C.S. 2 . .	c	c	c	+++	+	c	c	c	+++	+	trace	trace
C.S. 3 . .	c	c	+++	++	o	c	c	c	+++	+	+	trace
C.S. 5 . .	c	c	+++	++	o	c	c	c	c	++	+	o
C.S. 6 . .	c	c	c	+++	+	c	c	c	c	++	trace	o
C.S. 7 . .	c	c	c	+++	+	c	c	c	c	++	trace	o
C.S. 17 (1) .	c	c	c	+++	+	c	c	c	c	++	trace	o
C.S. 18 .	c	c	c	+++	+	c	c	c	+++	++	trace	trace
C.S. 21 .	c	c	+++	+	o	c	c	c	+++	+	+	trace
C.S. 24 .	c	c	c	+++	+	c	c	c	c	+	trace	o
C.S. 25 .	c	c	c	+++	+	c	c	c	c	+	trace	o
A 3 . .	c	c	c	+++	+	c	c	c	c	++	trace	o
A 4 . .	c	c	c	c	+	c	c	c	c	+	o	o
A 6 . .	c	c	c	c	++	c	c	c	+++	+	o	trace
A 9 . .	c	c	c	+	o	c	c	+++	++	o	trace	+
A 11 . .	c	c	c	c	+	c	c	c	c	o	o	o
A 12 . .	c	c	c	c	+	c	c	c	c	++	o	o
A 14 . .	c	c	c	c	+	c	c	c	+++	+	o	trace
A 15 . .	c	c	c	c	++	c	c	c	c	+	o	o
A 18 . .	c	c	c	+++	+	c	c	c	+++	+	trace	trace
A 17 . .	c	c	c	c	++	c	c	c	c	++	o	o
B 2 . .	c	c	+++	+	o	c	c	c	+	+	+	trace
C.S. 8 . .	++	o	o	o	o	o	o	o	o	o	c	c
A 7 . .	+++	o	o	o	o	++	o	o	o	o	c	c
C.S. 20 .	+	o	o	o	o	o	o	o	o	o	c	c
C.S. 4 . .	c	c	c	+++	+	c	c	c	c	++	trace	o
A 13 . .	c	c	c	++	+	c	c	c	c	++	trace	o
A 1 . .	c	c	c	+++	++	c	c	c	c	+	trace	o
A 2 . .	c	c	++	+	o	c	c	+++	+	o	+	+
A 16 . .	c	c	c	c	++	c	c	c	c	++	o	o
B 12 . .	c	c	c	+	+	c	c	c	+	o	trace	trace
B 3 . .	c	c	c	c	++	c	c	c	c	++	o	o
A 10 . .	c	c	c	++	+	c	c	c	c	+	trace	o
C.S. 14 .	c	c	c	c	++	c	c	c	c	++	o	o

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

unlike the type strain of the main group, P 1, is less "pure," since its serum agglutinates many of the latter group to almost the full titre. The same overflow of the agglutinating action to strains not belonging to the group was observed in connection with serum A 17, which, as has just been seen, seems as pure a type of the main Group I as does C.S. 8 for the C.S. 8 group, when absorption is resorted to instead of agglutination as the test.

So striking was this selective action in the absorption test with serum C.S. 8 that further experiments were done in which very large amounts of culture were employed, 80 mg. in one c.c. of 1–100 dilution of serum, to see whether protein-complexes capable of absorbing agglutinin were present at all in these related but not identical strains. No increase in absorption appeared, although very marked agglutination with abundant deposit had occurred in the absorbing mixture; the serum still agglutinated completely up to 1–1500 after absorption with all the main Group I strains except two (A 9 and A 12), with which slight absorption had taken place, agglutination being complete only up to 1000. The agglutinin for the strains themselves contained in serum C.S. 8, in most cases sufficient to give complete agglutination at 1–1000, had, however, completely disappeared after absorption.

What explanation can be given for this cross-agglutination without cross-absorption in the case of these two strains and sera C.S. 8 and P. 1? The following hypothesis would appear to meet the case. The two special antigens are present in both the strains C.S. 8 and P. 1; in the former strain the amount of special P. 1 antigen is small and in the latter the amount of special C.S. 8 antigen is small. In the sera produced by the two strains both agglutinins are present, the P. 1 agglutinin being, however, less in amount in C.S. 8 serum and the C.S. 8 agglutinin less in amount in P. 1 serum, so that when each serum is diluted to the full titre the agglutinating action of the "foreign" agglutinin in each case disappears. In consequence when C.S. 8 serum is absorbed by the strain P. 1 only the P. 1 portion of its total agglutinin is removed; but this portion is in any case non-effective at the full dilution so that no diminution in the activity of the C.S. 8 agglutinin is visible after absorption by P. 1. Exactly the same happens when P. 1 serum is absorbed by strain C.S. 8.

There remain eight strains related to Group I on the strength of agglutination tests, but excluded by negative absorption tests from both the groups just discussed.

Sub-Group (2).

Serum A 13, produced by one of these, agglutinates one other strain to the full titre and one to 1–1000, while most of the main Group I and the sub-group (1) strains are agglutinated up to 1–100 only. Ab-

sorption tests with this serum are summarised in Table VII and amply confirm the suspected existence of still another group. The technical details and symbols employed are in general the same as in Tables IV, V and VI.

TABLE VII.

Absorption of agglutinin from serum A 13.

Serum	Agglutination with Homologous Strain A 13				Agglutination with Related Strain C.S. 4				Absorption of Agglutinin for Homologous Strain A 13	Absorption of Agglutinin for Strain C.S. 4
Control unabsorbed	100	300	800	1200	100	300	800	1200		
	c	c	c	c	c	c	c	c		
Absorbed by Strain										
C.S. 1 . .	c	c	c	+	c	c	c	+ + +	trace	o
C.S. 17 (1) .	c	c	+ + +	+	c	c	c	+	+	trace
C.S. 8 . .	c	c	c	c	c	c	c	c	o	o
C.S. 20 .	c	c	c	+ +	c	c	+ + +	+ +	o	trace
C.S. 4 . .	+	o	o	o	o	o	o	o	c	c
A 13 . .	+	o	o	o	+	o	o	o	c	c
A 23 . .	c	+ + +	+ +	+	c	c	+ + +	+	+ +	+
A 1 . .	c	c	+ + +	+	c	c	+ +	+	+	+
A 2 . .	c	c	c	+	c	c	c	+	trace	trace
A 16 . .	c	c	+	o	c	c	o	o	+	+
A 10 . .	c	+	o	o	+ + +	o	o	o	+ + +	c
C.S. 16 .	c	c	c	c	c	c	c	c	o	o
*N 29 . .	+	o	o	o	o	o	o	o	c	c

* Naso-pharyngeal strain, see p. 231.

It will be seen that, of the twelve spinal strains tested, two only, the homologous strain A 13 and strain C.S. 4, give complete absorption, each absorbing the agglutinin for both itself and the other. In addition, strain A 10 gives well-marked absorption of the agglutinin for A 13 and complete absorption of that for C.S. 4, while strain A 23 absorbs the greater part of the agglutinin for A 13. All the others, including strains identified as belonging to the main Group I, the sub-group (1), the main Group II, and others unidentified but related by agglutination to Group I, give more or less definite but slight absorption only.

The results of absorption from serum A 13 are thus less sharply defined than those of the sera just discussed, in the sense that allied but not identical strains exist which also absorb agglutinin, but it is evident that the strains A 13 and C.S. 4 have been identified with one another and sharply distinguished from the groups hitherto established; while two other strains, A 10 and A 23, not hitherto identified with

any group, have been placed in close relationship with this sub-group (2), of which A 13 is the type.

Sub-Group (3).

In Table VIII, which follows, the results are given of absorption from the serum prepared by inoculation of this A 10. The technique and symbols employed are as in previous absorption experiments.

The results obtained are remarkable. In the first place, as might have been expected, the related strains of the previous group A 13 and C.S. 4, as well as the strain A 23, which agglutinated to the full titre with serum A 10 (*vide* Table I), all absorb completely, or almost completely, the agglutinin for the homologous strain. This confirms the deduction drawn from Table VII, namely, the close relationship of these four strains. Of the other strains employed, C.S. 1, a typical main Group I strain, and A 1 and A 2, allied to but not identical with this main group, all fail to absorb more than small amounts of the A 10 agglutinin. A 1, however, absorbs a marked amount of the agglutinin for the strain A 13, as also does A 16, which is another unidentified ally of the main Group I. These two, then, though only distantly related to A 10, have a connecting relative in the strain A 13.

Strains C.S. 16, C.S. 14, and A 24, on the other hand, are definitely members of Group II, being agglutinated strongly by the main Group II serum and not at all by the main Group I, while the sera they produce by injection into animals agglutinate Group II strains to a high titre and Group I strains slightly, if at all. Yet C.S. 16 absorbs the A 10 agglutinin almost, if not quite, as well as the strains C.S. 4, A 13, and A 23, which have been put down as closely related to A 10, while A 24 absorbs only a small amount of A 10 agglutinin and C.S. 14 almost none.

This is a case in which agglutination reactions evidently fail to agree with the results of absorption, since strain C.S. 16 agglutinates with A 10 serum up to 1–500 only (*vide* Table I), a level which is reached also by most of the strains in Table VII which show feeble, if any, absorption.

For the present, until the absorption results with the Group II sera have been discussed, it will be better simply to note this anomaly, without trying to explain it. But it may be observed at this point that the sera P 1, A 17, and C.S. 8, behave very differently from serum A 10 in the sharpness with which absorption differentiates individual strains. The former three either show practically complete absorption or practically none, whereas A 10 serum gives all degrees of absorption with

TABLE VIII.

Absorption of agglutinin from serum A 10.

Serum	Agglutination with Homologous Strain A 10						Agglutination with Related Strain A 13						Absorption of Agglutinin for Homologous Strain A 10	Absorption of Agglutinin for Strain A 13
Control unabsorbed	100	300	600	800	1000	1500	100	300	600	800	1000	1500		
	c	c	c	c	c	+ + +	c	c	c	c	c	+ + +		
Absorbed by Strain														
C.S. 1 . . .	c	c	c	c	+ +	+	c	c	c	c	+	o	+	+
C.S. 4 . . .	c	+ +	o	o	o	o	+ +	o	o	o	o	o	+ + +	c
A 13 . . .	+	o	o	o	o	o	o	o	o	o	o	o	c	c
A 23 . . .	+ +	o	o	o	o	o	c	+ +	o	o	o	o	c	+ + +
A 1 . . .	c	c	c	c	+ + +	+ +	c	+ + +	+ +	+	o	o	+	+ + +
A 2 . . .	c	c	c	c	+ + +	+ +	c	c	c	+ + +	+ +	+ +	+	+ +
A 16 . . .	c	c	c	+	o	o	c	c	+ + +	+	+	o	+ +	+ + +
A 10 . . .	o	o	o	o	o	o	+	o	o	o	o	o	c	c
C.S. 16 . .	+ +	o	o	o	o	o	+ + +	+ + +	o	o	o	o	c	c?
C.S. 14 . .	c	c	c	c	+ + +	o	c	+ +	+	o	o	o	+	+ + +
A 24 . . .	c	c	c	+ + +	+ + +	+	c	c	c	+ + +	+ + +	+	+ +	+ +
*N 4 . . .	+	o	o	o	o	o	o	o	o	o	o	o	c	c

* Naso-pharyngeal strain, see p. 231.

different strains. Further, as has been noted, in the case of the former sera increase in the amount of bacterial protein used for absorption does not increase the amount of agglutinin removed by the poor absorbers. With serum A 10 increase in the amount of culture to 80 mg. per c.c. of 1–50, or repeated addition of smaller amounts, increases the amount of absorption in the case of certain strains, *e.g.* A 24, but not in others, *e.g.* C.S. 14. This quality is characteristic of two of my Group II sera.

The strain A 10 is one of those given me by Dr Arkwright. It came from one of the first cases in the epidemic which broke out among Canadian troops in this country, and has been described already by Dr Arkwright (1915)[1] under the name of "Murray"; in his description he records that it belonged serologically to the meningococcus species, and not to the parameningococcus of Dopter. Translated into the terms of my classification, this means that it belonged to Group I, and not to Group II. In my hands, although it inclines to the main Group I on its agglutination reactions, and is linked up to it by the relations shown by absorption, it has evidently affinities in addition which connect it with Group II.

There are thus several considerations all leading to the conclusion that A 10, whether originally or as the result of subsequent variation, lies intermediate between the main Group I and the main Group II, for

(1) it agglutinates equally, though relatively feebly, with the sera of both groups;

(2) it does not absorb agglutinin from either of the two main group sera, but

(3) the serum which it produces agglutinates indifferently, though weakly, strains of both main groups, and

(4) picks out, as agglutinating to high titre, a strain allied to Group I (A 13), and also one allied to Group II; while

(5) its agglutinin is absorbed both by strains allied to Group I and by at least one typical Group II strain; and

(6) the behaviour of the serum on absorption with different quantities of culture resembles that of other sera prepared with Group II strains.

Sub-Group (4).

Example of an aberrant type.

The absorption tests with serum A 2 may be summed up shortly without a table. None of the other spinal strains absorb more than small amounts of A 2 agglutinin, even when added in great excess, and the

[1] *Brit. Med. Journ.*, II. 885.

Group II strains in particular are completely devoid of absorptive capacity; indeed, after absorption with large amounts of these the titre of the "absorbed" serum appears slightly higher than before, the agglutination in the highest dilutions being more complete. Strain A 2, therefore, remains alone among my known pathogenic strains as the type of its sub-group, but it will be seen later that this sub-group is well represented among naso-pharyngeal meningococci.

As has been noted, it agglutinates indifferently with sera of both Group I and Group II, but to a low titre with both. It absorbs small amounts of agglutinin from certain Group I sera, but none, or practically none, from the Group II. It has, therefore, been put among the strains which lean towards the main Group I. With strain A 2 a serum of high titre was obtained after prolonged immunisation, agglutinating it completely at 1–1500. No other strain was agglutinated to the same level with this serum, but among spinal strains one, B 12, gave almost complete agglutination at 1–1000; two strains, C.S. 4 and A 13, which belong to one group, as has been seen, were agglutinated completely at 1–500, while C.S. 20, belonging to another group, and A 15, which was identified with the main Group I, almost reached the same titre. The others of the main Group I gave complete agglutination at 1–100, but only traces of agglutination at 1–500. The Group II strains were either completely negative, or gave only traces of agglutination at 1–100.

The agglutinogenic characters of A 2 thus confirm in the main its position as allied to Group I, but show that it possesses a pronounced individuality, distinguishing it from all the other spinal strains in my possession.

Absorption tests with serum A 2 confirm its peculiarities; none of the spinal strains, not even those agglutinating relatively well, remove any but the merest traces of the homologous agglutinin, although removing all the agglutinin for themselves. It must be noted further that the other strains hitherto unidentified, strains A 1, A 16, B 12, B 1, B 3, B 5 and B 10, fail to disclose definite relationship with A 2 as the result of application of its serum.

These strains may resemble A 2 in possessing serological properties peculiar to each, or they may belong to entirely different groups. It would be necessary, in order to settle more definitely their relationship, to investigate their agglutinogenic action, but even this, as has been seen in connection with A 2, and as will be seen with the naso-pharyngeal strains, might leave the question in doubt.

Sub-Group (5).

The three strains placed together in this sub-group are those which, though showing by their agglutination that they incline definitely towards the main Group I, all fail to absorb more than small amounts of agglutinin from the sera of this main group and the sub-group sera. Two of them, however, have been identified by absorption tests with a naso-pharyngeal strain which itself is similarly inclined to the main Group I, but also fails to absorb. This sub-group (5), then, seems to consist of two sub-groups, the second consisting solely of the strain B 12, which, like A 2, is highly individual in its characters.

Main Group II.

The results of absorption with the main Group II serum, P 2, as has been mentioned, have not the uniformity which is typical of the main Group I. In Table IX, subjoined, an example is given of the irregularity often met with. The quantity of culture employed in each case was 15 mg. for each c.c. of 1–50 dilution of the serum; this is the minimum for complete absorption of agglutinin by the homologous strain for itself, but does not reach the minimum which certain strains require to remove completely the agglutinin, either for themselves or for the homologous strain. Hence some of the strains in this table which fail to remove the agglutinin completely appear as incomplete absorbers only because, not 15 mg., but, say, 30 mg., of culture were required to complete the removal.

But it will be seen that those strains, numbers (1) to (7), which have already been identified with other groups are in agreement in removing traces at most of the homologous agglutinin. Similarly, four of the five strains which, in Table I, were shown to agglutinate feebly with all the sera, and were hence not capable of classification (Nos. 9, 10, 11, 12 here), fail to absorb definite amounts of agglutinin.

On the other hand, of the twenty strains put in the main Group II on the strength of agglutination, eight give absorption equal, or almost equal, to the homologous strain, nine give absorption just short of complete, while three give definite absorption but leave intact perhaps half the agglutinin for the homologous strain.

Of these three partial absorbers one agglutinates to full titre with serum P 2, while two agglutinate completely at 1–1500 (*vide* Table I).

Some indication of sub-grouping within Group II appears on inspection of the column recording absorption of agglutinin for C.S. 14.

Table IX.

Absorption of agglutinin from serum P 2.

Serum	Agglutination with Homologous Strain P 2					Agglutination with Related Strain C.S. 14					Absorption of Agglutinin for Homologous Strain P 2	Absorption of Agglutinin for Strain C.S. 14
Control unabsorbed	100 c	600 c	1000 c	1400 c	2000 + + +	100 c	600 c	1000 c	1400 c	2000 + + +		
Absorbed by Strain												
(1) C.S. 1 .	c	c	c	c	+	c	c	c	+ + +	o	trace?	+
(2) C.S. 17 (1)	c	c	c	c	+	c	c	c	+	o	trace?	+
(3) C.S. 25 .	c	c	c	c	+ + +	c	c	c	+ + +	o	o	+
(4) C.S. 8 .	c	c	c	c	+ + +	c	c	c	c	+ +	o	o
(5) C.S. 20 .	c	c	c	c	+ +	c	c	c	+ +	+	o	+
(6) C.S. 4 .	c	c	c	c	+ +	c	c	c	+ +	+	o	+
(7) A 23 .	c	c	c	+ +	+	c	c	+ +	+	o	+	+ +
(8) A 16 .	c	c	c	c	+	c	c	c	+ + +	o	trace?	+
(9) B 1 .	c	c	c	+ + +	+	c	c	+ +	+	o	+	+
10) B 3 .	c	c	c	c	+ +	c	c	c	c	+ +	o	o
11) B 5 .	c	c	c	c	+	c	c	c	o	o	trace?	+
12) B 10 .	c	c	c	c	+ +	c	c	c	c	+ +	o	o
13) A 10 .	c	c	c	+ + +	+	c	c	+ +	+	o	+	+
14) A 24 .	c	+ + +	+ +	o	o	+	o	o	o	o	+ + +	c
15) A 22 .	c	+ +	o	o	o	o	o	o	o	o	+ + +	c
16) C.S. 9 .	c	+ + +	o	o	o	+ + +	o	o	o	o	+ + +	c
17) C.S. 10 .	+ + +	+	o	o	o	o	o	o	o	o	c	c
18) C.S. 11 .	c	+ +	o	o	o	o	o	o	o	o	+ + +	c
19) C.S. 12 .	c	+	o	o	o	o	o	o	o	o	+ + +	c
20) C.S. 14 .	c	c	+ + +	+ +	o	o	o	o	o	o	+ +	c
21) C.S. 15 .	+ +	+	o	o	o	o	o	o	o	o	c	c
22) C.S. 16 .	o	o	o	o	o	o	o	o	o	o	c	c
23) C.S. 17 (2)	c	c	+ + +	+ +	+	c	c	c	+ + +	+	+ +	+
24) C.S. 19 .	c	c	+ + +	+ + +	+	+ +	o	o	o	o	+ +	c
25) C.S. 22 .	+ +	o	o	o	o	+ + +	o	o	o	o	c	c
26) C.S. 26 .	+ + +	+	o	o	o	+	o	o	o	o	c	c
27) A 20 .	+ + +	+ +	o	o	o	+	o	o	o	o	c	c
28) A 25 .	c	c	+ + +	+	o	+ +	o	o	o	o	+ +	c
29) B 4 .	c	+ + +	+	+	o	c	+ +	o	o	o	+ + +	+ + +
30) B 6 .	c	+ + +	o	o	o	c	+	o	o	o	+ + +	+ + +
31) B 7 .	+ + +	+	o	o	o	+ + +	+	o	o	o	c	c
32) B 8 .	c	+ +	o	o	o	c	+	o	o	o	+ + +	+ + +
33) B 9 .	+ +	+	o	o	o	c	+	o	o	o	c	+ + +
34) B 11 .	c	+ +	o	o	o	c	+	o	o	o	+ + +	+ + +
35) P 2 .	+ +	+	o	o	o	+	o	o	o	o	c	c

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

This strain is itself one of the partial absorbers although a good agglutinator; if, in serum P 2, the agglutinin responsible for the agglutination of C.S. 14 were in some way different from the main agglutinin produced by P 2, then strains which differed from P 2 in the same way as C.S. 14 does, ought to remove completely the agglutinin for C.S. 14, but to a varying and less extent for P 2. And, in fact, strain C.S. 19, which absorbs P 2 agglutinin only "+ +," absorbs C.S. 14 agglutinin completely, while many of the strains absorbing P 2 just short of completely, remove all the agglutinin for C.S. 14.

The indication is that C.S. 14 is allied to C.S. 19, and that both differ slightly from P 2 and most of the other strains, but the evidence is slender, as will be seen later.

One strain removes agglutinin only partially for both C.S. 14 and P 2; this is C.S. 17 (2), the variant of the Group I strain, C.S. 17, already referred to as having changed its character. One strain, B 8, which, on agglutination, appeared a doubtful adherent of Group II, is shown by absorption to be closely allied to, if not identical with, the main group.

The absorptive action of strains C.S. 14, C.S. 16 and A 24 should be specially noted, as sera have been prepared with them, the behaviour of which will be recorded below. Of these C.S. 16 and A 24 are good absorbers, while C.S. 14, as just remarked, is a partial absorber.

In Table X are given the results of absorption from serum C.S. 14, the quantity of culture used being 10 mg. per c.c. of 1–50 serum dilution.

In the first place it may be noted that the first five strains, which belong to Group I, absorb, at most, traces of agglutinin, as do also the next two strains, B 1 and B 3, which were doubtful adherents of Group II. Strain A 10, the strain intermediate between the two groups, also removes only a minute amount of C.S. 14 agglutinin.

On the other hand fifteen strains, which by agglutination were put in Group II, absorb all or almost all the agglutinin both for C.S. 14 and for P 2, the type strain of Group II. Among these are C.S. 19 and C.S. 17 (2) which showed only partial absorption with P 2 serum (*vide* Table IX).

No well-marked difference can be detected between the absorption of agglutinin for the homologous strain C.S. 14 and that for the type strain P 2.

In Table XI are given the results of absorption from serum C.S. 16 by a selection of Group II strains, with, as controls, three strains more

closely allied to Group I and the four strains which, on agglutination, were referable to neither group but inclined, perhaps, to Group II.

It will be seen that all these controls absorb, at most, traces of agglutinin for both the test emulsions, while the strains already put in Group II absorb the agglutinin for both, either completely or almost completely. It is worth noting that strain A 10 which, as was seen in

TABLE X.

Absorption of agglutinin from serum C.S. 14.

Serum	Agglutination with Homologous Strain C.S. 14				Agglutination with Related Strain P 2				Absorption of Agglutinin for Homologous Strain C.S. 14	Absorption of Agglutinin for Strain P 2
	100	500	1000	1500	100	500	1000	1500		
Control unabsorbed	c	c	c	c	c	c	c	c		
Absorbed by Strain										
C.S. 1	c	c	c	++	c	c	c	+++	trace	trace?
C.S. 17 (1)	c	c	c	c	c	c	c	c	o	o
C.S. 8	c	c	c	c	c	c	c	c	o	o
C.S. 4	c	c	c	+++	c	c	c	++	trace?	trace
A 23	c	c	+++	+	c	c	c	c	+	o
B 1	c	c	+++	+	c	c	++	+	+	+
B 3	c	c	c	c	c	c	c	+++	o	trace?
A 10	c	c	+++	+	c	c	c	c	+	o
A 24	o	o	o	o	o	o	o	o	c	c
A 22	+	o	o	o	o	o	o	o	c	c
C.S. 9	+	o	o	o	o	o	o	o	c	c
C.S. 10	++	o	o	o	c	o	o	o	c	+++
C.S. 11	o	o	o	o	o	o	o	o	c	c
C.S. 12	+++	+++	+	o	++	+	o	o	c?	c
C.S. 14	o	o	o	o	+	o	o	o	c	c
C.S. 15	+	o	o	o	o	o	o	o	c	c
C.S. 16	++	o	o	o	o	o	o	o	c	c
C.S. 17 (2)	c	+	o	o	+++	o	o	o	+++	c
C.S. 19	+++	o	o	o	o	o	o	o	c	c
C.S. 22	+	o	o	o	o	o	o	o	c	c
C.S. 26	+	o	o	o	o	o	o	o	c	c
A 20	o	o	o	o	o	o	o	o	c	c
A 25	o	o	o	o	o	o	o	o	c	c
P 2	o	o	o	o	o	o	o	o	c	c

Table VIII, produced a serum from which C.S. 16 absorbed practically all the agglutinin, fails entirely to absorb agglutinin from C.S. 16 serum; on repeating this absorption test with A 10 on C.S. 16 serum, but using 60 mg. of culture per c.c. in three instalments of 20 mg. instead of 10 mg. in all, the negative result persisted, as was the case also with strains C.S. 20 and A 13 similarly tested.

Sub-Group (6).

Finally the results of absorption of agglutinin from serum A 24 may be given (Table XII). Strain A 24, as has been remarked (*vide* Tables I, IX, X, and XI), agglutinated to the full titre with all the Group II sera and absorbed their agglutinin completely, but the agglutination reactions of the serum it produced indicated that it possessed peculiar characters,

TABLE XI.

Absorption of agglutinin from serum C.S. 16.

Serum	Agglutination with Homologous Strain C.S. 16				Agglutination with Related Strain A 22				Absorption of Agglutinin for Homologous Strain C.S. 16	Absorption of Agglutinin for Strain A 22
Control unabsorbed	100	400	800	1200	100	400	800	1200		
Absorbed by Strain	c	c	c	c	c	c	c	c		
C.S. 20 .	c	c	c	c	c	c	c	c	o	o
A 13 . .	c	c	c	c	c	c	c	c	o	o
A 10 . .	c	c	c	c	c	c	c	c	o	o
A 24 . .	+ + +	+	o	o	+ +	+	o	o	c	c
C.S. 10 .	+ + +	+	o	o	+	o	o	o	c	c
C.S. 14 .	+ + +	+	o	o	+ +	o	o	o	c	c
C.S. 16 .	+ +	o	o	o	+	o	o	o	c	c
C.S. 19 .	+ + +	+	o	o	+ +	o	o	o	c	c
A 20 . .	+ + +	+	o	o	+ + +	o	o	o	c	c
A 25 . .	c	+ + +	o	o	+ + +	+	o	o	+ + +	c
B 1 . .	c	c	c	+ + +	c	c	c	c	trace?	o
B 3 . .	c	c	c	c	c	c	c	+ + +	o	trace?
B 4 . .	+ + +	o	o	o	+	o	o	o	c	c
B 5 . .	c	c	c	+ + +	c	c	c	c	trace?	o
B 6 . .	c	o	o	o	+	o	o	o	+ + +	c
B 7 . .	+ + +	o	o	o	+	o	o	o	c	c
B 8 . .	o	o	o	o	trace	o	o	o	c	c
B 9 . .	c	o	o	o	+	o	o	o	+ + +	c
B 10 . .	c	c	c	c	c	c	c	c	o	o
B 11 . .	c	o	o	o	+	o	o	o	+ + +	c

not revealed by these sera, since only one of the other Group II strains appeared to be specifically affected.

The technical details of the experiment are as before, but the symbols have been given a higher value in consequence of the low titre of the serum, complete absorption being presumably easier from a weak serum.

The peculiar characters of A 24 and A 22 are confirmed by the absorption test and justify the position of these two strains as forming

a sub-group within the main Group II. The strain A 22, which, as was shown in Table I, was unique in reaching the full titre for this serum, removes, as might be expected, all the agglutinin for A 24, while one other strain, C.S. 11, which agglutinated with A 24 serum up to 1–500 only, absorbs almost all the agglutinin for both A 24 and A 22. Strain C.S. 9, which also agglutinated up to 1–500, absorbs perhaps half the agglutinin, while C.S. 22, which had the same agglutination titre, absorbs none of the A 24 agglutinin and only a small amount of that for A 22.

The type strain, P 2, absorbs none of the A 24 agglutinin, in spite of the fact that A 24 absorbs the P 2 agglutinin from P 2 serum almost completely.

TABLE XII.

Absorption of agglutinin from serum A 24.

Serum	Agglutination with Homologous Strain A 24				Agglutination with Related Strain A 22				Absorption of Agglutinin for Homologous Strain A 24	Absorption of Agglutinin for Strain A 22
Control unabsorbed	100 c	400 c	800 c	1000 c	100 c	400 c	800 c	1000 c		
Absorbed by Strain										
C.S. 9	+ + +	+ + +	+ +	o	c	+ + +	o	o	+ +	+ +
C.S. 11	+ +	trace	o	o	+ +	o	o	o	c	c
C.S. 22	c	c	c	c	c	+ + +	o	o	o	+ +
C.S. 14	c	c	c	c	c	+ + +	trace	o	o	+ +
A 22	o	o	o	o	o	o	o	o	c	c
A 24	o	o	o	o	o	o	o	o	c	c
A 25	c	c	+ + +	+ + +	c	+ + +	o	o	+	+ +
P 2	c	c	c	c	c	c	+ + +	o	o	+

Sub-Group (7).

The sub-group (7) which was created on the strength of agglutination results and recorded in Table III has by the use of the absorption test had its numbers reduced from five to four, since the strain B 8 although agglutinating relatively feebly has been shown to absorb all the agglutinin from serum C.S. 16. The other four strains, however, remain as a sort of scrap-heap since though they certainly agglutinate rather better with the Group II sera than with Group I yet their agglutination can in no case be said to be decisive, and they absorb practically none of the various agglutinins tested. They may belong to one group or may each represent strains of high individuality; they may be compared in this respect to the strain A 2 and may bear the same relation to the main Group II that this strain has shown towards the main Group I.

General Behaviour of Group II Sera on Absorption.

The results of absorption from the Group II sera are evidently much more complicated and difficult to explain than those with the Group I sera.

In the first place there is the phenomenon, which was noted in connection with serum A 10, that increase in the amount of culture used for absorption may transfer a strain from the category of poor absorbers into that of complete absorbers. This is even more marked in the case of the Group II sera P 2 and C.S. 14. For example, with serum P 2 in one absorption experiment, using 8 mg. of culture per c.c. of 1–50 dilution, the homologous strain P 2 and strains C.S. 16, A 22, C.S. 22, out of fifteen Group *II* strains, were the only ones which reduced the titre to just below 1–500; with C.S. 9, C.S. 10, C.S. 11, C.S. 12, A 20, A 24, A 25, C.S. 14 and C.S. 19 the titre was reduced to incomplete at 1–1000 only; on repeating the extraction with the same amount of culture on the partially exhausted serum, the titre was reduced somewhat below the levels shown in each case in Table IX, *e.g.*, with P 2, the homologous strain, only traces of agglutination persisted at 1–100, as also with A 22, C.S. 22, C.S. 10, C.S. 11, C.S. 12 and A 20, while with C.S. 9, A 24, A 25 and C.S. 14 agglutination was still complete at 1–100 but incomplete at 1–500. If an arbitrary point had been chosen as determining a positive result, as, for example, complete or incomplete agglutination at 1–500, this dilution only being tested, and if the test had been performed only after the first extraction, very definite grouping might have appeared, P 2, C.S. 16, A 22, C.S. 22 forming one group among the Group II strains, while the others might have been regarded as doubtful allies. When more opportunity for absorption was provided by increasing the amount of extracting material, the results, as shown in Table IX, indicate that many more strains are capable of removing completely, or almost completely, the homologous agglutinin.

Exactly similar results have been obtained with serum C.S. 14; as with serum P 2, increase in the amount of culture used in the absorption test broadened the selective action of the agglutinin to a quite remarkable extent.

This behaviour makes it extremely difficult to use the absorption test in estimating the relationships of the different strains of Group II. Some indication of the presence of sub-groups is to be found in the relative ease with which certain strains remove agglutinin as compared with others, but it is very doubtful if any stress can be laid on this, since, if all the agglutinin in the test serum can be combined and removed by a strain, even though large quantities of culture are necessary, the negative controls remaining unaffected, it shows, at least, that the partial absorption given by the smaller amount does not depend on the presence in the serum of an anti-body which fits the homologous and identical strains but does not fit those absorbing less readily. And the demonstration of an anti-body which is incapable of absorption by non-identical strains is the admitted criterion in distinguishing "specific" from "group" agglutination.

Yet the existence in the Group II sera of such "group" agglutinins would seem to be demanded by the fact that they agglutinate and are absorbed to a small extent by strains such as those of the main Group I and some of its allies. The difference is that increase in the amount of culture used does not lead with these, as with the less active absorbers among Group II strains, to increased absorption. These strains which fail to absorb even when present in great excess act as controls to show that

in the action of large amounts of culture physical non-specific destruction of agglutinin is not responsible for absorption.

On what, then, depends the less easy absorption by strains which, in actual biochemical constitution, are apparently identical with the strain producing the serum?

To answer this would require a much more profound knowledge than is in existence of the factors at work in the absorption test, and it has not been found practicable at this time to investigate these factors as a problem apart from the classification of pathogenic meningococci and the identification of naso-pharyngeal strains with them.

The following considerations may apply. The absorption of agglutinin by a suspension of bacteria may be conceived as a simple chemical combination, each coccus, for example, being capable of combining with a certain definite maximum of the protein in the serum which carries the agglutinating property. This maximum might vary in different strains, so that a strain in which it was high would remove more agglutinin for a given number of cocci than another in which it was low, although the qualitative nature of the chemical action taking place did not differ. This supposition would account for most of the phenomena in the absorption tests with Group II strains, were it not for the difficulty caused by the fact that a strain may have a low maximum with one serum and a high maximum with another, though capable of removing all agglutinin from both.

An alternative conception depends on the phenomena observed in biological precipitation. When, for example, dilute horse serum is mixed with the serum of a rabbit immunized against horse protein, a bulky precipitate is thrown down, by far the greater part of which is derived, not from the dilute horse serum, the precipitinogen, but from the anti-serum, the precipitin. The same observation has been made with bacterial precipitation, such as occurs when a filtered extract of bacteria has been brought in contact with the corresponding anti-serum. Here the amount of the bacterial protein has no direct relation with the amount of protein thrown out of solution and removed from the anti-serum.

There is considerable evidence that bacterial precipitin and bacterial agglutinin are attached to one and the same protein in the anti-serum, but, even if attached to different proteins, the production of a precipitin-precipitum in the mixture would necessarily produce a mechanical agglomeration and deposit of the suspended cocci which would both simulate and take part in the total agglutination. Hence, supposing, as seems reasonable, that dissolved bacterial proteins, as well as intact cocci, are present in the suspensions, then not only agglutination but removal of agglutinin would depend both on deposition of cocci which had combined with agglutinin and on the precipitation of large quantities of anti-body in consequence of the presence of dissolved bacterial protein acting as a precipitinogen.

Hence one might account for the different behaviour of Group I and Group II strains in absorption tests by supposing that Group I strains more readily liberate their protein into solution, so that more of this second method of removing anti-body occurs; while Group II strains might depend for agglutination and for agglutinin-absorption on the much less powerful action of direct combination of cocci with agglutinin.

I have some evidence that suspensions of those strains which remove agglutinin on the addition of small amounts of culture contain more detached soluble protein than those which require large amounts, since I have repeatedly seen with Group I

strains, as contrasted with Group II strains in which the phenomenon is much less marked, that when a suspension of culture has been freed from all intact cocci by the centrifuge or prolonged standing, or by filtration, the clear liquid produces almost as large a precipitum with the anti-serum as the same fluid containing the suspended cocci; this precipitum must consist chiefly of the anti-body present in the serum, the removal of which probably depends on the precipitinogen contained in the dissolved bacterial protein and is thus governed by laws different from those ruling the absorption by intact cocci.

On this hypothesis, then, the different behaviour of certain strains and sera as regards the facility of absorption would be explained by the varying amount of dissolved protein present in the suspensions—(this would explain also the difference between individual Group II strains). A correlated factor may be the varying extent to which anti-body capable of behaving like precipitin is present in the serum—(this would still further explain the difference in behaviour between sera which respond to increase of culture by increase of absorption and those which do not).

But in reviewing generally the difficulties in reconciling the different behaviour of meningococcus strains and anti-sera, I can only repeat that, until the essential nature is known of the physico-chemical action which results in absorption of agglutinin, there is little probability of satisfactorily explaining the apparent anomalies.

Two of these anomalies, which have been specifically mentioned and are of similar character, are (1) the fact that strain A 24 absorbs completely from serum C.S. 14 the agglutinin for C.S. 14, while C.S. 14 removes none of the specific agglutinin for A 24 from serum A 24, and (2) that strain C.S. 16 removes all, or nearly all, the specific agglutinin for strain A 10 from serum A 10, but strain A 10 removes little or none from serum C.S. 16.

It is just possible that the specific agglutinin in A 24 serum consists almost entirely of very specialised anti-bodies, corresponding to very specialised antigens in strain A 24 and hence not affected by strain C.S. 14, while in serum C.S. 14 the anti-bodies present are of a more general character, each antigen in the strain having exerted an approximately equal effect on the animal. In the absorption of serum C.S. 14 by A 24 the antigens of general Group II character alone come into action, and the more special C.S. 14 anti-bodies, if any, are in such small amount that, though left in the serum after absorption by A 24, they only produce agglutination of low titre for C.S. 14.

A similar explanation in the other case meets with the difficulty that serum A 10 undoubtedly contains very special anti-bodies of predominating Group I character as well as the more general, and it is hard to conceive that strain C.S. 16 which, agglutinogenically, had little relation to A 10, contained enough of the special corresponding antigen to affect these appreciably, whereas serum C.S. 16 resembles very much serum C.S. 14 and would, therefore, contain special anti-bodies in less amount than the more general anti-bodies; it should hence be affected appreciably by contact with the general antigens of A 10.

The only explanation possible is that strain C.S. 16 at the time it was used for absorption had become modified in character, acquiring some of the characters more typical of A 10. That such modification may occur is to my mind probable, and the following section records variations in specific character as determined by absorption tests.

Variations in absorptive quality and capacity.

Variations in agglutinability have already been noted and illustrated in Table II. In particular, one strain, C.S. 17, was stated to have changed from agglutination to full titre with Group I serum when first isolated to agglutination to full titre with Group II serum after keeping in culture for a month, the other group serum in each case being almost if not quite negative. One month later it again agglutinated to full titre with the Group I serum and only slightly with Group II. This latter condition has persisted unchanged now for several months, but another sub-culture from that which gave Group II agglutination has remained equally definitely of Group II character in agglutination; the former I have designated C.S. 17 (1), the latter C.S. 17 (2). Both have been plated out at intervals of about a month and have given on each of three occasions practically pure cultures of their own agglutinating type, four colonies being investigated from each plate. One colony from C.S. 17 (2), however, agglutinated to 1–500 with serum P 1, the Group I serum, as well as to full titre with serum P 2, the Group II serum, and therefore might be regarded as tending towards an intermediate position.

From the Group I sera P 1 and A 17 the strain C.S. 17 (1) absorbs practically all the agglutinin for the homologous strains as for itself, while C.S. 17 (2) removes none either for the homologous strains or for C.S. 17 (1). From the Group II sera P 2 and C.S. 14 the strain C.S. 17 (1) absorbs no agglutinin either for the homologous strains or for C.S. 17 (2), whereas the latter absorbs rather less than half the agglutinin for the homologous strains (noted as + +), but all the agglutinin for itself.

From serum C.S. 8 both C.S. 17 (1) and C.S. 17 (2) remove no agglutinin for the homologous strain, but C.S. 17 (1) removes all the agglutinin for itself (titre before absorption 1–500), while C.S. 17 (2) leaves the agglutinin for C.S. 17 (1) intact.

From serum A 10, C.S. 17 (1) removes agglutinin entirely for itself, and "+ +" for the homologous strain, while C.S. 17 (2) removes none of either agglutinin.

The absorption tests thus confirm the Group I condition of C.S. 17 (1), but leave doubtful the exact position of C.S. 17 (2); yet by agglutination this strain is definitely of Group II, and this is confirmed by its agglutinogenic action, since a serum prepared with C.S. 17 (2) agglutinated all the Group II and left practically unaffected the Group I strains.

The conclusion to be drawn is either that this strain C.S. 17 changed its serological character entirely during cultivation, or that in the

original infection two strains of meningococci were present, one or other predominating at different times during cultivation until in separate instances one or other died out entirely. On the whole I feel inclined to take the latter view, but have felt bound to record the occurrence as a possible instance of profound variation in the serological character of a meningococcus strain.

Strain A 24 has been particularly variable in the extent of its absorbing powers for certain sera. At one time 10 mg. of its culture removed only traces of the homologous agglutinin from serum P 2, while three months later the same quantity removed all the homologous agglutinin, although in the two cases the cultures were equally well agglutinated by serum P 2. But even with its own serum such irregularities in absorptive power appeared. Sub-cultures from two colonies on the same plate differed entirely in their absorptive power, one removing all the A 24 agglutinin, while the other barely affected this serum, although again both agglutinated well. On the other hand, with serum C.S. 16, these two colonies, though differing markedly in agglutinability, one being complete at 1–1500, while the other did not agglutinate above 1–100, absorbed the C.S. 16 agglutinin completely in both instances.

Strain C.S. 16 absorbed from serum A 10 on November 11, 1916, only traces of agglutinin, whereas later, on February 21, 1917, it absorbed A 10 serum completely, although used in exactly the same amount and under the same conditions.

Finally, the strain C.S. 10 may be mentioned. In July, 1916, it absorbed C.S. 14 serum completely as it did again in August, but in November it had become much less agglutinable and also failed entirely to absorb C.S. 14 agglutinin. Since then it has remained inagglutinable with all the sera tested and removes no agglutinin from any of the Group II sera. Morphologically and culturally it is unchanged.

Explanation of Serological Differences.

Before proceeding to discuss the naso-pharyngeal strains it may be well at this point to summarise my argument as to the reason for the differences found by serological tests in different strains of meningococci obtained from spinal fluid, leaving over for the moment the question of the validity of these differences as criteria for classification into fixed types.

I have concluded, then, that peculiarities in the protein molecule affect the antigenic action of the different strains and excite in the

immunised animal the production of anti-bodies of similar peculiarity, that in some strains, *e.g.* those within or allied to Group I, this particularisation of the protein molecule is much more advanced than in others, *e.g.* Group II; in the former, serological identity is much more striking when it appears and serological difference is similarly more obvious, while in the latter the peculiar properties are in the background, and the general action of the protein as antigen and combining factor in serological reactions is more pronounced, so that peculiarities in the strains belonging to this group are readily concealed by the interaction of their more general characters. It is probable that peculiarities limited to small sub-groups of strains, or even to individuals, also exist in Group II, but, as a result of this quantitative difference in particularisation, the sub-groups among the former are well marked, while in the latter they are submerged in the general character of the large group[1].

These conclusions certainly seem to explain the experimental facts. The question remains whether these peculiarities are permanent features of the strains or only temporary, however strong they may be. Possibly they have been acquired during a long process of evolution, and hence deserve consideration as stages in the direction of specific differentiation; or they perhaps merely express variations recently acquired as the result of interaction with the tissues of their host.

Unfortunately, conclusive evidence on these points is extremely difficult, if not impossible, to get. In artificial culture the variations which I have been able to demonstrate are not conclusive evidence that in nature change can take place from one group to another, and are open to the objection that the original strain may not have been pure but contained two varieties existing side by side, but predominating at different times.

The relative proportions of the different types isolated from the human host in health and disease are, however, somewhat suggestive of modification due to environment, and this question will be referred to again when the serological reactions of naso-pharyngeal strains have been discussed.

Naso-pharyngeal Strains.

As has already been indicated, these strains were isolated from naso-pharyngeal mucus, and each represents a colony found on plates of

[1] This difference between the groups may depend, however, on the accident of choice of strains used for producing sera; it seems possible that, with a different selection, sera of Group I might be found containing agglutinins in large amounts for its sub-groups, and sera of Group II (cf. serum A 24) containing agglutinins acting only on small sub-groups.

Kutscher's medium inoculated with this material. Morphologically and culturally they were indistinguishable from the meningococci isolated from cerebro-spinal fluid in cases of the specific disease. Sixteen represent the survivors of the thirty strains from Lambeth outpatients discussed in my former report; the remainder, making up the total of seventy-one, were isolated during the first half of 1916, chiefly from soldiers in camp or garrison.

Agglutination and Absorption Reactions.

In the following table (Table XIII) the dilutions are given at which complete agglutination was produced by the two sera, C.S. 14 and C.S. 16, with all the naso-pharyngeal strains in my possession. These sera were both prepared with strains of pathogenic origin, both belonging to Group II; the titre for the homologous strain was in each case 1–1500. In the same table the results of absorption of the agglutinin for the homologous strains are given for these two sera, using 10 mg. of culture with each strain for 1 c.c. of the serum diluted 1–50; the symbols have the same value as in the previous absorption tables for these sera (Tables X and XI).

It will be seen that, as with the spinal strains, the two sera have much the same agglutinating properties, the differences observed being of a minor quantitative character. Taken together they agglutinate to the full titre 30 of the 71 strains, while nine others are agglutinated to 1–1000 and five to 1–500 with one or other serum. This leaves 27 strains which on agglutination results are excluded from the group represented by C.S. 14 and C.S. 16, and already defined as Group II.

The results of absorption are closely comparable: 37 strains remove completely the agglutinin for the homologous strain from one or both the sera, while three others remove it almost completely, the effect being noted as + + + which means, as before, that agglutination of the homologous strain by the serum after absorption was complete at 1–100 but incomplete at 1–500. These 40 strains include the 30 reaching the full agglutination titre, the nine reaching 1–1000 and one of the five reaching 1–500.

With the remaining 31 strains absorption was either entirely negative (seven strains) or reduced the titre of the serum only to a slight degree.

The conspicuous feature of the table is thus the large number of strains, 56 per cent., which can be identified with one or both of the two strains of spinal origin, this identification depending on the combining properties of the strains in question with the anti-bodies produced by

TABLE XIII.

Naso-pharyngeal strains: results of agglutination and absorption with two Group II spinal sera.

Strain	Agglutination with Serum C.S. 14	Agglutination with Serum C.S. 16	Absorption from Serum C.S. 14	Absorption from Serum C.S. 16	Strain	Agglutination with Serum C.S. 14	Agglutination with Serum C.S. 16	Absorption from Serum C.S. 14	Absorption from Serum C.S. 16
N 1	100	100	tr.	tr.	N 37	100	500	o	+
N 2	tr.	tr.	tr.	tr.	N 38	1500	1500	c	c
N 3	tr.	100	tr.	tr.	N 39	1500	1500	c	c
N 4	100	100	+	tr.	N 40	1500	1000	c	c
N 5	100	500	+	+	N 41	1500	100	c	c
N 6	1500	1000	c	c	N 42	1000	1000	+ + +	c
N 7	tr.	100	tr.	tr.	N 43	1500	1500	c	c
N 8	o	100	tr.	tr.	N 44	1500	1500	+ + +	+ + +
N 9	1500	1000	c	+ + +	N 45	1500	1500	c	c
N 10	tr.	100	tr.	o	N 46	1500	1500	c	
N 11	tr.	o	o	o	N 47	1000	1500	+ + +	c
N 12	tr.	100	o	o	N 48	100	100	+	o
N 13	100	100	+ +	+	N 49	100	100	o	+ +
N 14	tr.	o	o	o	N 50	1000	1000	c	c
N 15	1500	100	+ + +	+ +	N 51	1500	1500	c	c
N 16	tr.	100	+ +	+	N 52	1000	1000	c	c
N 17	500	1500	+ + +	+	N 53	o	o		o
N 18	100	100	+	o	N 54	1000	1500	c	
N 19	o	o	o	o	N 55	1500	1500	+ + +	c
N 20	100	100	tr.	o	N 56	1000	1500	c	+ + +
N 21	1500	1000	c	+ + +	N 57	1500	1500	c	
N 22	1000	1500	c	c	N 58	o	100	tr.	+
N 23	tr.	o	tr.	tr.	N 59	1500	500	c	c
N 24	100	100	+		N 60	1500	1500	c	c
N 25	1500	1500	c		N 61	1500	1500	c	c
N 26	100	o	tr.	+	N 62	1000		c	
N 27	1500	1000	c		N 63	1000	1000	c	c
N 28	1500	1000	c	+ + +	N 64	o	o	o	
N 29	o	o	o	o	N 65	100	100	tr.	
N 30	100	o	o	+	N 66	500	500	o	tr.
N 31	1000	1000	c		N 67	100	100	o	tr.
N 32	1500	1500	c	c	N 68	o	500	o	tr.
N 33	1500	1500	c	c	N 69	500	500	c	c
N 34	1000	1000	c	c	N 70	1500	1000	c	c
N 35	1000	1000	c	c	N 71	1000		c	
N 36	500	1000	c	c					

the known pathogenic meningococci. This predominance of Group II strains applies both to contacts and non-contacts, and in this connection it is of great interest to remember that in the collection of 60 known pathogenic strains only 21, 35 per cent., were identified with this group,

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

while 33, 56 per cent., were either identified with, or shown to be nearly related to Group I.

There remain among the 71 naso-pharyngeal strains 31 which have still to be identified.

Among these, ten have been specifically identified with spinal strains belonging to Group I and its immediate allies, while six strains have given evidence of relationship but not identity. Table XIV summarises the reasons for the specific identification or relationship of these Group I naso-pharyngeal strains under the headings of (1) agglutination with sera prepared with spinal strains of the group, (2) absorption of agglutinin from these sera, (3) agglutinogenic action in the case of eight strains showing that the sera produced by them agglutinate certain spinal strains, and (4) absorption of agglutinin from these sera by these spinal strains. Information on the last two points has not been collected in the case of all strains, since the labour involved in preparing and testing sera with all would probably not have given results of proportionate value.

The sub-groups referred to in the column headed "*Absorption of Group I agglutinin and consequent sub-grouping*" are those found among spinal strains and recorded in Table III.

On examining this table it will be seen that only one strain, N 19, has been found identical with the main Group I, one strain, N 1, with the sub-group (1) represented by C.S. 8, one strain, N 29, with the sub-group (2), of which A 13 is the type, three strains, N 4, N 5, and N 13, with the closely-related sub-group (3), of which A 10 is the type spinal strain, while four, N 2, N 10, N 58, and N 67, have been identified with the sub-group (4), represented by strain A 2, which, though allied to the main Group I, is highly specialised, and has no near relative among my other spinal strains. One strain, N 48, is of much interest as being closely related to, if not identical with, four of the spinal strains which could not be identified by the use of any of the spinal sera, though two of them possessed definitely stronger affinities for Group I, and were hence put in sub-group (5), while two were indifferent to both the main group sera, and were put in sub-group (7), the "scrap-heap." In addition four strains, N 3, N 7, N 8, and N 11, have been shown to possess relationship with the spinal strain A 2, but on evidence insufficient for the presumption of specific identity. One of these, N 7, is similarly related to at least one of the main Group I strains, A 12, the relationship in common being apparently some quality of a minor nature in the case of A 12, since it is insufficient to differentiate A 12 from others of the main group.

TABLE XIV.

Serological relationship and classification of naso-pharyngeal strains allied to Group I.

Strain	Agglutination with Group I Sera	Absorption of Group I Agglutinin and consequent sub-grouping	Agglutinogenic Properties, *i.e.* Reactions of Serum prepared with Strain: Agglutination of Spinal Strains	Absorption with same Spinal Strains
N 1	Serum P 1, 500 ,, C.S. 8, 1000	Serum P 1, + ,, C.S. 8, c Sub-group (1)	[Full Titre, 1500] Main Group I, 100 C.S. 8 Group, 1000 Main Group II, o	+ c o
N 2	Serum P 1, 100 ,, C.S. 8, tr. ,, A 2, 800	Serum P 1, o ,, C.S. 8, o ,, A 2, c Sub-group (4)	[Full Titre, 1000] Main Group I, tr. C.S. 8 Group, tr. A 2, 600 Group II, o	o o c o
N 3	Serum P 1, tr. ,, C.S. 8, 0 ,, A 2, 500	Serum P 1, o ,, C.S. 8, o ,, A 2, + + Related to sub-group (4)	*Not tested*	*Not tested*
N 4	Serum P 1, 500 Sera C.S. 8 and A 2, 100 ,, A 13 and A 10, 1000	Serum P 1, o Sera C.S. 8 and A 2, o ,, A 13 and A 10, c Sub-group (3)	,,	,,
N 5	Serum P 1, 100 ,, A 10, 500	Serum P 1, tr. ,, A 10, + + + Sub-group (3)	,,	,,
N 7	Serum P 1, 500 ,, A 2, 500	Serum P 1, o ,, A 2, + Related to sub-group (4)	[Full Titre, 800] Main Group I, 300 A 2, 800 Group II, 300	tr. [A 12 = + + +] + + o
N 8	Serum P 1, 500 ,, A 2, 500	Serum P 1, o ,, A 2, + Related to sub-group (4)	*Not tested*	*Not tested*
N 10	Serum P 1, 500 ,, C.S. 8, 0 ,, A 2, 500	Serum P 1, + + ,, C.S. 8, o ,, A 2, + + + Sub-group (4)	[Full Titre, 1000] Main Group I, 500 Group C.S. 8, tr. A 2, 1000	+ o + + +
N 11	Serum P 1, 100 ,, C.S. 8, 100 ,, A 2, 500	Serum P 1, o ,, C.S. 8, o ,, A 2, + Related to sub-group (4)	*Not tested*	*Not tested*
N 13	Serum P 1, tr. ,, C.S. 8, 500 ,, A 10, 500	Serum P 1, o ,, C.S. 8, + ,, A 10, + + + Sub-group (3)	[Full Titre, 1500] Main Group I, 500 Group C.S. 8, 100 ,, A 10, 1000 ,, II, 100	o o + + + o
N 19	Serum P 1, 1500 ,, C.S. 8, 100	Serum P 1, c ,, C.S. 8, tr. Main Group I	[Full Titre, 1500] Main Group I, 1000 C.S. 8, 100	c [A 17 = + +] o

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

TABLE XIV *continued.*

Strain	Agglutination with Group I Sera		Absorption of Group I Agglutinin and consequent sub-grouping		Agglutinogenic Properties, *i.e.* Reactions of Serum prepared with Strain: Agglutination of Spinal Strains		Absorption with same Spinal Strains
					[Full Titre, 1000]		
N 29	Serum P 1,	500	Serum P 1,	tr.	Main Group I,	100	+
	,, C.S. 8,	500	,, C.S. 8,	o	Group C.S. 8,	o	o
	,, A 13,	1500	,, A 13,	c	,, A 13, A 10,	1000	c [A 10 = + + +]
			Sub-group (2)				
					[Full Titre, 1000]		
N 48	Serum P 1,	100	Serum P 1,	+	Main Group I,	100	+
					Group A 13,	500	+ +
			? Sub-group (5)		Unidentified B 3, B 10, A 1, A 16,	1000	+ + +
					Group II,	100	o
N 58	Serum P 1,	100	Serum P 1,	o	*Not tested*		*Not tested*
	,, A 2,	500	,, A 2,	+ + +			
			Sub-group (4)				
N 67	Serum P 1,	100	Serum P 1,	o	,,		,,
	,, A 2,	500	,, A 2,	+ + +			
			Sub-group (4)				

In the case of one strain, N 18, not included in the table, the evidence of relationship to Group I is confined to the single fact that it agglutinates much better with Group I than with Group II sera. Absorption tests, however, fail to identify it with any spinal strain, and its agglutinogenic action has not been ascertained owing to its extreme toxicity for rabbits.

There remain fifteen naso-pharyngeal strains which resemble the spinal strains from cases in children, B 1, B 5, B 3, and B 10, in their poor reaction with all the spinal sera tested.

Eleven of these, N 12, N 14, N 16, N 20, N 26, N 37, N 49, N 64, N 65, N 66, and N 68, agglutinate completely up to 1–500 with serum P 2, but absorb insignificant amounts of P 2 agglutinin (*vide* Table XIII). They show at most traces of agglutination with the various Group I sera, and, like the spinal strains referred to above, cannot be definitely brought into relationship with each other or with any of the other spinal strains. Their nearest allies, to judge by agglutination, are the Group II strains; perhaps sera prepared with the more divergent members of this group might identify these unclassified spinal and naso-pharyngeal strains, but in default of such sera they must all be placed as *incertae sedis*.

Of the remaining four strains, N 53, N 30, N 23 and N 24, *strain N* 53, the strain isolated from a soldier who had been pronounced to be a "chronic carrier," similarly unidentified by absorption, agglutinates

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

completely at 1–100 but not higher with sera P 2 and A 2, fails to absorb from both these sera, and neither agglutinates nor absorbs agglutinin with the others; its position in the meningococcus category is thus still more indefinite. *Strain N* 30, however, though agglutinating feebly or not at all with the spinal sera, is strongly agglutinated by the serum prepared with N 1, and absorbs almost all its agglutinin; but N 1 has been shown to conform to the tests of specific identity with the spinal strain C.S. 8, so that strain N 30, though itself divergent from this spinal strain, evidently possesses a protein-molecule resembling to a large extent that of the strain N 1; and this molecule, as has been seen, can carry combining properties of high valency for the C.S. 8 group serum: N 30, therefore, is linked up to the sub-group (1) through the intermediary strain N 1. Similarly, *strains N* 23 *and N* 24, though agglutinating relatively feebly with spinal sera, agglutinate up to 1–800 with the serum prepared with strain N 48, and absorb most of its agglutinin. This N 48 serum, as has been noted, agglutinates to full titre, and is absorbed by, certain strains of spinal origin which themselves were unidentified by the use of spinal sera, and were hence put in the "scrap-heaps," sub-group (5) or sub-group (7).

Had I prepared sera with such spinal strains they might have resembled serum A 2 in the peculiar restriction of their specific properties, and, like it, might have picked out and identified with themselves naso-pharyngeal strains such as N 23 and N 24, which, in absence of the appropriate spinal serum, can be brought into relationship with strains of known pathogenicity only in virtue of their relationship to another naso-pharyngeal strain N 48.

Relative Proportions of the Different Groups.

(*a*) *In cerebro-spinal and naso-pharyngeal strains.*

In my collection of the former, 55 per cent. were allotted to Group I and of these almost three-fourths were of one type as determined by absorption tests, and this was therefore called the main Group I. Among the naso-pharyngeal strains only 22 per cent. were related to Group I, and of these only 6 per cent. belonged to the main group of this, while 25 per cent. belonged to the rare sub-group (4), of which A 2 is the type: the other sub-groups (2), (3) and (5) having, as among the spinal strains, one or two members each.

On the other hand (as I have already mentioned), the main Group II included 56 per cent. of the naso-pharyngeal strains and only 35 per

cent. of those of spinal origin, while 8·5 per cent. of the spinal strains and 21 per cent. of the naso-pharyngeal remain ungrouped.

It has been suggested in discussing spinal strains (p. 228) that the difference in relative proportions of the different groups may indicate that the serological qualities of the different members may not depend on fixed characters, but are subject to modification as the result of their environment. It might seem from the above statistics that the Group II condition of the meningococcus was more characteristic of it while sojourning in the naso-pharynx, and that in that region modification in the direction of Group I rarely reached the full degree as represented by the main group of the Group I spinal strains, but that it halted at the stage represented by the sub-groups of Group I, which are less distantly related to Group II. It is equally possible that residence in the naso-pharynx tends to modify Group I strains in the direction of Group II.

But the most important indication furnished by a comparison between the groups found in the two classes is that each serological type found among the strains of pathogenic origin is represented among those from the normal naso-pharynx, and it seems probable that, if a sufficient number of pathogenic strains were examined for comparison, all the naso-pharyngeal strains would find an identical type among these.

In 58 of my 71 naso-pharyngeal strains sufficient serological similarity exists to permit of identification with strains of cerebro-spinal origin by complete or well-marked absorption of the agglutinin fitting these, while only 13 remain in which relationship is suggested by agglutination but has not been confirmed by absorption. The complete identification of these 13 strains might involve prolonged search for spinal strains similarly aberrant and the production of many sera with these before the exact type of aberration in each naso-pharyngeal strain met its prototype among those of spinal origin. It is evident, I think, that, given the necessary time and material, for any type of coccus in the naso-pharynx possessing the morphological and cultural characters of the meningococcus a counterpart may be found, possessing its more or less peculiar serological reactions, among cultures from the meninges.

(*b*) *As regards age of patient and severity of disease produced.*

In 36 of my 60 spinal strains I know the age of the patient from whom the strain was cultivated. Of these, 25 were of 13 years or under, while 12 were over 13. Of the 25 strains from children, 9 were classed as

Group I and 16 as Group II, while of the 12 adult strains 9 were of Group I and 3 of Group II. Though these are small figures from which to draw deductions, there is some indication that Group II affects children more than adults.

Twenty cases recovered and 8 died, the fate of the others being unknown to me. Of the cases which recovered 14 belonged to Group I and 6 to Group II, while 2 Group I cases died and 6 Group II. One cannot argue from this that Group II strains produce the severer infections; my results are probably due to their greater incidence on infants. This greater incidence of Group II on infants may indicate that the infection of children is frequently acquired from normal adults who, as has been shown, are apt to harbour Group II strains more than Group I.

Scientific and Practical Value of Serological Tests.

(*a*) *Absorption.*

I have now described more or less fully the serological reactions of 131 strains, of which 60 were of known pathogenicity, while 71 had shown no evidence of capacity for producing disease in the particular host.

In the first place, the value of these reactions in differentiating groups within the broad meningococcus category requires consideration. It has been seen that on the strength of agglutination two main groups are well defined: members of each group agglutinate strongly with the sera prepared by inoculation of members of the same group but feebly or not at all with sera prepared from members of the other group.

But on subjecting these main groups to more elaborate serological tests differences appear among strains which on simple agglutination would at first sight be grouped together. These differences already begin to reveal themselves when careful estimation is made of relative agglutinability with different sera and are still more marked when the power of absorbing agglutinin is taken into account. In many cases, indeed, these differences in absorptive character are absolute, *i.e.* there is either complete absorption, indicating perfect adaptation of the bacterial receptors towards the agglutinin, or complete absence of absorption, showing that the special combining parts of the protein molecule for the particular agglutinin are entirely lacking.

By some authorities[1], as I mentioned before, such differentiation is regarded as sufficient for the erection of species among strains belonging

[1] Bainbridge and O'Brien (1911), *Journ. of Hygiene*, xi. 68.

to the Food-Poisoning Group which are otherwise indistinguishable from each other. There some support is given to such an attitude by the differences in pathogenic action said to be shown by the proposed species, the one being associated with continued fever and the other with acute enteritis. But in the case of the meningococcus there is little evidence of difference in the disease produced by the different groups, and the question arises, What is the significance of the limitation to certain strains of the power of absorbing agglutinins? Is one justified in saying that the capacity for absorbing each other's agglutinin, which is found to be the common property of certain strains but absent from others, is sufficient to define the former as fixed types or species?

The final answer to such questions cannot, of course, be given until bacteriologists agree as to the criteria necessary for the erection of species among bacteria. But if it is agreed that this character, the absorption of each other's agglutinin, defines strains as belonging to a fixed type or species, then the number of such fixed types or species of meningococcus must be taken as very large, since, even in the small collection of strains which I have been able to study, there appear on this basis at least eight of them. It follows that in the placing of an unknown strain in relation to its proper type on this principle of classification at least eight monovalent sera must be applied. Yet even eight sera would fail to classify by absorption all the meningococci found producing disease.

Further, although in many cases the special power of absorbing a certain agglutinin appears sharply and definitely peculiar to certain strains, in others it appears to depend on quantitative rather than on qualitative differences, and the presence or absence of absorptive capacity is much less sharply defined. For example, in the case of strains C.S. 4, A 13, A 23, and A 10 absorption tests might lead to each being given the status of a separate type or to all being put together according as large or small quantities of culture were employed in the reaction.

Added to this difficulty, there is a serious element of confusion arising from the fact, already noted, that the same strain may vary in absorptive capacity on different occasions. Even with the main groups doubt may arise as to the type to which a particular strain should be assigned: for example, the Group II strain C.S. 16 has been seen to absorb agglutinin completely, not only from all the typical Group II sera, but also from the Group I serum A 10.

The conclusion, I think, should be that peculiarities in the absorption of agglutinin are not of sufficient permanence and are not sufficiently sharply defined in all cases to permit of their employment in the creation of hard-and-fast types or species. If they are investigated and determined with all the accuracy possible, it is evident from the number of distinct sub-groups which I have demonstrated in my small collection of strains that a useless multiplicity of types would be created; if rougher tests are depended on, the different types shade into each other and no boundary line can be drawn.

But in the meantime the chief interest of these elaborate serological reactions is the practical one. Can one by their use distinguish a meningococcus of pathogenic origin from other cocci morphologically and culturally identical?

There can be no doubt that the positive demonstration of mutual absorptive capacity for each other's sera proves sufficiently the serological identity of two strains; and such proof involves the admission that where one of the two is known to have caused disease, the other, whatever its origin, must be capable, under the proper conditions, of doing the same. Even when absorptive capacity has been demonstrated only for one strain with the serum of the other, *i.e.* where a nasopharyngeal strain absorbs the agglutinin from a spinal serum, it is probable that the same proof and admission may be allowed.

But it does not at all follow that the failure to demonstrate such identity by absorption tests excludes the coccus of unknown pathogenicity from the category of possible pathogenic strains. For it is evident, even in my small collection, that differences sufficient to prevent identification with each other are already numerous among the known pathogenic meningococci, and the probability of success in such identification is in direct proportion to the number of different sera used.

To take a concrete example, suppose the known pathogenic strains A 7 and C.S. 20 had been submitted for diagnosis as being of doubtful pathogenicity, and suppose, as might easily have happened since the group to which it belongs forms only 5 per cent. of my total, that the serum C.S. 8 had not been prepared or used, then these two strains would have been tested in vain against at least four different sera; if the positive result of an absorption test had been regarded as essential, they would have remained as doubtful meningococci or might even have been excluded altogether.

Hence the practical attitude towards the absorption test for the identification of unknown strains with pathogenic meningococci should

be that a positive outcome is decisive, that a negative result means nothing, and that even when this latter repeats itself with several different sera it does not exclude the strain from possessing pathogenicity.

(*b*) *Agglutination.*

Although the absorption test is too precise in its action and might exclude not only strains from the naso-pharynx of indeterminate serological character, but also known pathogenic strains, it remains for discussion whether simple agglutination might not furnish a criterion distinguishing naso-pharyngeal strains of common occurrence among non-contacts from those of spinal origin. Simple agglutination is less precise in its action and not confined as in the case of the absorption test to strains exactly identical with the strain producing the serum. Some authorities maintain that by simple agglutination a rough line could be drawn including the great majority of the pathogenic strains and excluding a large proportion of the strains commonly occurring in the normal naso-pharynx, and that it constitutes, therefore, a most useful criterion for practical use in determining the danger to be attached to meningococcus carriers.

This is the view taken by Colonel Mervyn Gordon in his recommendations for the control of cerebro-spinal fever in the Army[1], his reasoning, I think, being that naso-pharyngeal strains which fail to reach the full agglutinin titre with any of his four types of serum prepared from pathogenic strains are to be distinguished from strains of epidemiological significance.

My results indicate that there are many more than four agglutination types among pathogenic meningococci, since I have found at least eight and even then have left some unidentified.

It might theoretically be possible, however, to collect and classify a representative series of pathogenic strains and to prepare with them a manageable set of sera, to one of which, at least, any meningococcus of cerebro-spinal origin would respond in positive fashion; and it would make the task easier if abnormal strains occurring in sporadic cases and among infants could be ignored. In such circumstances a coccus from the naso-pharynx which failed to agglutinate satisfactorily might be put down either as not pathogenic or as belonging to a type producing disease so rarely that it escaped collection, and hence must be of no epidemiological importance.

[1] *Medical Research Committee, Special Report Series*, No. 3, p. 7.

But against this is the difficulty that variations in agglutinability, as has been shown, are very great and might easily lead to the exclusion of pathogenic strains if one test only were applied; indeed, with the naso-pharyngeal strains in particular, agglutinability is often much less pronounced in the first sub-culture than in succeeding ones.

Still more important as affecting the use of naso-pharyngeal swabbing in the control of cerebro-spinal fever is the prevalence in the normal naso-pharynx of meningococci of typical serological character. This is so great that the most elaborate investigation of naso-pharyngeal strains could only exclude quite a minor proportion of the total found; the great majority are identical with types of pathogenic strains of frequent occurrence in the epidemic disease and exclusion of the few which remain unidentified with my pathogenic strains would not modify the epidemiological problem represented by the large percentage of positive findings which I have demonstrated among normal persons.

Prevalence of Naso-pharyngeal Meningococci in the Various Groups of Persons examined.

In my first report on this subject I showed that among the out-patients attending Lambeth Infirmary in June and July, 1915, 22 per cent., 30 out of 138 examined, although they had had no relation to cerebro-spinal fever, harboured in their naso-pharynx micro-organisms indistinguishable from the meningococcus in morphology and culture, while in 13·7 per cent., 19 out of the total, the serological reactions of the strains isolated confirmed their meningococcal nature. During May, 1915, 56 school children attending a rural school in Kent were similarly examined with negative results, one strain only being found which on cultural and morphological characters resembled the meningococcus; it failed, however, to respond to the serological tests applied.

In the present report additional material in the form of naso-pharyngeal strains has been collected, and these as well as the strains previously described have been submitted to more elaborate serological examination.

On January 20, 1916, 20 children, infants under seven years attending an urban school, were examined. Again only one suspicious strain was found; it failed to agglutinate with any serum, and died out before further tests could be performed.

During the four months February to May, 1916, I had, in the course of my public health duties in a small area in North-East Kent, an opportunity of investigating the carrier percentage among soldiers and of

comparing the results found where cerebro-spinal fever had occurred with those among men who had had no connection with the disease. Omitting the school children, I have thus a set of strains from civilians (Lambeth) dating from 1915, and seven sets of soldiers, a total of 142, dating from 1916, each set forming a sample of a different body of men but in each case of men living in the conditions of close association found in camp or barracks.

The general result has been to confirm the conclusion arrived at in my first report that meningococci are to be found in a considerable percentage of persons in whom no relation to cases of cerebro-spinal fever is discoverable. In the case of the Lambeth population the percentage, as revised in the light of more elaborate study of the strains, and on the basis of full serological identification, is 11·6 per cent., while among the soldiers the percentage on the same basis varied from 10·5 per cent. to 57·6 per cent. among those in whom relation to the disease was either absent or remote, and from 25·9 per cent. to 37·5 per cent. among those in direct contact with cases.

In the following table (Table XV) the various points of importance in connection with each set are given for comparison, together with the percentages of persons in whom meningococci were cultivated from the naso-pharynx.

In analysing this table it will be best to discuss first each set *seriatim* and to reserve the general considerations to the end.

In the first set, the Lambeth out-patients, out of 138 individuals, 30, or 22 per cent., furnished strains culturally identical with the meningococcus, 19 of which, making 13·7 per cent. of the total, were also similar serologically.

Of these 30 strains 16 have been submitted to more elaborate serological tests, the rest having accidentally perished in the interval.

The following are the results of this reinvestigation:

	On more elaborate tests	
In First Report	Complete identity	Incomplete proof of identity
Identified by agglutination 11 strains . . .	8 strains	3 strains
Identified by culture 5 strains . . .	1 strain	4 strains
16	9	7

As these 16 strains have not been selected in any way, the remainder having been eliminated by accident during sub-culture, they may be considered as fairly representing the original 30; calculated on this

TABLE XV.

Meningococcus carriers in the different population groups.

No. of group	Description of group and relation to whole population	Date examined	Relation to cerebro-spinal fever before examination	No. examined	Percentage with meningococci identified completely by absorption tests	Percentage including meningococci which show relationship by absorption tests but not identity	Percentage including meningococci agglutinating with anti-meningococcus sera but not absorbing	Relation to cerebro-spinal fever after examination
					per cent.	per cent.	per cent.	
1	Lambeth Out-Patients (revised statistics)	June 1–July 15, 1915	None demonstrable	138	11·6	16	22	None known
2	Soldiers, Garrison A of 500 men	Feb. 17, 1916	None	20	20	25	35	None
3	Soldiers in huts, Battalion at M. of 2000	March 2, 1916	None	19	10·5	15·5	21	2 cases in Battalion 3 months later
4	R.A.M.C. staff of Battalion at T.	March 23, 1916	2 cases in connection with Battalion 1 week before, not direct	5	40	60	60	None
5	Soldiers of Battalion at T. of 1500 men	March 28, 1916	The above 2 cases, but not direct	19	57·6	57·6	63·2	None
6	Six soldiers, 2 civilians in billets in Town S.	March 20 (4)–April 5 (4), 1916	Direct contacts of cases	8	37·5	37·5	37·5	None
7	Soldiers in huts of Battalion at M. as in (3)	May 11, 1916	None	18	33·3	33·3	38·9	2 cases, not in same huts, but in Battalion on May 24
8	Soldiers in huts of Battalion at M. as in (3) and (7)	May 26, 1916	2 cases in same huts, direct contacts	54	25·9	25·9	33·3	None

TOTALS.

Civil	Group 1	1915	Non-contact	138	11·6	16	22
Military	Groups 2, 3, 7	1916	Non-contact	57	21	24·5	31·5
	Groups 4, 5	1916	Doubtful indirect contacts	24	54	58	62·5
	Groups 6, 8	1916	Direct contacts	62	27	27	34

https://doi.org/10.1017/S0022172400007130 Published online by Cambridge University Press

proportion the percentages of persons harbouring meningococci of the same serological types as those found in cerebro-spinal fever is reduced to 11·6 per cent., as shown above. None of the 138 persons examined had had any connection with cases of meningitis as far as careful inquiry could determine, and no case of cerebro-spinal fever has occurred to my knowledge among them since.

The next group, No. 2, dates from February 17, 1916, about nine months later, and consists of 20 soldiers collected in four batches of five from different rooms in garrison barracks. The total strength of the garrison at the time was about 500 men; the sample taken, though small, was, I think, fairly representative. There had been no case of cerebro-spinal fever in the garrison for at least two years.

Four of these men, one out of each batch of five, gave cultures of typical meningococci, serologically identical with types apparently common in the epidemic disease; the percentage is thus 20 per cent. If the strain is included which showed serological relationship but not identity, the percentage rises to 25 per cent., while if all cases are included in which strains culturally identical were found the percentage becomes 35 per cent.

The next group in chronological order, Group 3, consisted of 19 men (20 were swabbed, but from one man both plates were overgrown with contaminating organisms, so that he is excluded from the set). These were swabbed on March 2, 1916, and came from soldiers of four different training companies living in huts in and near a coast village in Kent. Some 2000 soldiers in all were encamped there, and no case of cerebro-spinal fever had occurred among them since the construction of the camp. Two of these men were found to be carrying typical meningococci; from two others other two strains were cultivated, one of which showed definite relationship to pathogenic strains by absorption, while the other could not be identified thus although it agglutinated to some extent with specific sera. The percentage of positive carriers on the first basis is thus 10·5 per cent., on the second 15·5 per cent., and on the third 21 per cent.

The next group, Group 4, was examined on March 23, and consisted of five men on duty in the medical inspection room attached to a training battalion of about 1500 men in camp in a rural parish in Kent, but about a mile from a small town.

Two cases of cerebro-spinal fever had occurred in connection with this battalion one week before; one case was a soldier belonging to it who developed the disease and died while away on leave; the other was

an infant living in a small house where two of the soldiers were billeted. Indirect connection probably occurred between the contacts of these cases and the medical staff, though none of an intimate character, as far as is known.

Two of the five were found to be carrying typical meningococci, while a third yielded a strain showing definite relationship by absorption tests; the percentage of carriers was thus 40 per cent. on the first basis and 60 per cent. on the second.

Group 5 consists of 19 men of the battalion just described. They came up for vaccination, being recent recruits, on March 28, 1916, and the opportunity was taken to examine their naso-pharynx. They had been living for a fortnight under canvas, but not all in the same tent. Their relation to cases of cerebro-spinal fever is similar to that described in connection with the previous group but more remote. Eleven of the 19 men were found to be carrying typical meningococci, and a twelfth yielded a strain related by agglutination reactions but failing to absorb. On the first basis 57 per cent. were carriers, on the second 63 per cent. A month later I examined the carriers again and found in every case meningococci of the same typical character.

Groups 6, 7 and 8 were all connected with cases of cerebro-spinal fever either immediately before or after swabbing, as contrasted with those hitherto described in whom connection with this disease was either absent or remote. In Groups 6 and 8 swabbing was done after direct connection with a case had been established; in Group 7 two cases occurred a fortnight after though not in direct connection.

Group 6 consists of contacts in billets of two cases in a small town in Kent, both soldiers, but of different battalions, the contacts consisting of three soldiers and one civilian in each case. The first case occurred on March 20, the second on April 5, the contacts of each being examined the following day. Two of the contacts with one case and one with the other were found to be carrying typical meningococci indistinguishable by absorption tests from pathogenic strains and from the 11 strains found in the previous group. The percentage on the total of eight contacts is thus 37·5 per cent. No restrictive measures were adopted in the case of one, a civilian; the soldiers were isolated by the military authorities concerned. No other cases appeared in the town during 1916.

Group 7 is drawn from the same battalion as Group 3, but consists of 18 different men living under the same conditions in the same place and collected from eight different companies. They were swabbed on May 11, ten weeks after the first batch described under Group 3. No

cerebro-spinal fever had occurred in the interval. Six of the 18, or 33 per cent. yielded typical meningococci, and one other gave an atypical strain which agglutinated up to 1–500, but failed to absorb agglutinin. If this one is included the percentage of carriers is 39 per cent.

The contacts living in the same two huts as two cases of the disease constitute Group 8, and were examined two days after the appearance of the disease. None of these men had come into the previous selections made for Groups 3 and 7. Out of the 54 contacts 14 yielded typical meningococci, a percentage of 26 per cent., while four others gave strains which agglutinated somewhat feebly with specific sera and failed to absorb agglutinin. If these four are included, the total carriers amount to 33·3 per cent.

SUMMARY AND CONCLUSIONS.

(1) Meningococci from 60 cases of cerebro-spinal fever have been submitted to serological tests and compared with meningococcus-like micro-organisms from the naso-pharynx of 71 normal persons. In these serological tests 19 monovalent anti-meningococcus sera were employed, of which 11 were prepared with meningococci of known pathogenic origin and eight with strains isolated from the naso-pharynx. The tests comprised careful estimation of the agglutinability of these 131 strains with the 19 different sera and also of the extent to which these strains absorbed and removed the agglutinin from one or more sera.

(2) The simple agglutination reactions effected a rough sub-division of these 131 strains into two groups, Group I and Group II, and indicated that in Group I there was a main group comprising the majority and at least five smaller groups each comprising a few strains only, while in Group II again a main group appeared comprising the majority and at least two smaller groups containing a few strains only.

(3) Tests for absorption of agglutinin confirmed this rough sub-division into two groups and distinguished with much greater precision the different main groups and smaller groups. In general, each of these groups was found to differ in that they did not absorb the agglutinin for members of the others, and members of each produced sera from which members of the others similarly failed to absorb agglutinin.

(4) Two of the smaller groups, however, represent two "scrap-heaps" of strains unidentified by absorption reactions but suggesting by their agglutination reactions that they belonged to Groups I and II respectively. In the former there were placed three strains of cerebro-spinal and one strain of naso-pharyngeal origin, and in the latter four strains of the former origin and thirteen strains of the latter.

(5) The other main groups and smaller groups contained representatives of both cerebro-spinal and non-contact naso-pharyngeal strains, though not in equal numbers: in the main group of Group I there were placed 24 spinal strains and only one non-contact naso-pharyngeal; in the main group of Group II there were placed 16 spinal strains and 40 naso-pharyngeal, of which 23 were from non-contacts and 13 from contacts.

(6) Variations in agglutinability and absorptive capacity were shown to be so great as to interfere seriously with the use of serological tests for identifying meningococci in practice.

(7) An additional difficulty affecting both the identification of meningococci and their classification into types or groups and sub-groups is that, even with seven sera, each corresponding to a different group, strains were found, both spinal and naso-pharyngeal, which failed to react typically with any and therefore could neither be ascribed to a particular type or group nor be identified on serological grounds with the other pathogenic strains.

(8) Hence it was concluded (*a*) that it is impossible to regard these types or groups as representing distinct classes limited by hard and fast lines, and (*b*) that it is unsafe to exclude any strain from possible pathogenicity on the ground of its failure to agree serologically with any of such sets of sera as are likely to be available in practice, since even the large series I employed failed to include all pathogenic strains.

(9) The conclusion in my first report is therefore maintained that any strain possessing the admitted morphological and cultural characters of the meningococcus should be regarded as potentially pathogenic without considering its serological reactions.

(10) I have found, however, that the strains obtained from the majority of carriers show by absorption tests complete serological identity with known pathogenic strains. To this criterion 58 of my 71 naso-pharyngeal strains conform. These were distributed as follows: 16 out of 138 out-patients attending Lambeth Infirmary in June and July, 1915 (11·6 per cent.); 12 out of 57 non-contact soldiers in February, March and May, 1916 (21 per cent.); 13 out of 24 soldiers in March, 1916, who had had no direct connection with cerebro-spinal fever but in whose neighbourhood two cases had occurred (54 per cent.); 17 out of 62 soldiers who had been in direct contact with cases of the disease (27 per cent.).

(11) Inclusion of those persons who were found to be carrying strains not fully identified serologically did not raise the percentage of carriers to a significant extent.