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UNIVERSITY OF EDINBURGH.

THESIS submitted for

DEGREE OF M.D.

by

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"IDENTIFICATION OF THE MENINGOCOCCUS IN CASES OF CEREBROSPINAL FEVER OCCURING ON TRANSPORTS CALLING AT CAPE TOWN, AND IN THE CAPE PENINSULA, SOUTH AFRICA, FROM JUNE 1916 to OCTOBER 1917."

January 1st, 1920.

IDENTIFICATION OF THE MENINGOCOCCUS IN CASES OF CEREBROSPINAL FEVER OCCURRING ON TRANSPORTS CALLING AT CAPE TOWN, AND IN THE CAPE PENINSULA, SOUTH AFRICA FROM JUNE 1916 TO OCTOBER 1917.

SUMMARY OF CONTENTS.

IDENTIFICATION OF THE MENINGOCOCCUS IN CASES OF CEREBROSPINAL FEVER OCCURING ON TRANSPORTS CALLING AT CAPE TOWN, AND IN THE CAPE PENINSULA, SOUTH AFRICA, FROM JUNE 1916 TO OCTOBER 1917.

The occurrence of Cerebrospinal Fever on Australian and other Transports calling at Cape Town was a matter of concern both to the Military and Civil authorities, especially as no cases of this disease had occurred among the civil population for some years.

Arrangements were made for Bacteriological examinations of cases and Carriers to be performed by me, the work being done in the Government Bacteriological Laboratory, by kind permission of Lt.-Col. G.W. Robertson, Government Bacteriologist, and Officer Commanding No. 3 Laboratory S.A.M.C.

1. ROUTINE PROCEDURE. All transports are inspected by the Port Health Officer and by a Military Medical Officer, who, upon finding any cases suspicious of Cerebrospinal Fever, report by phone to the Senior Military Medical Officer and to the Government Health Officer, who inform me. I proceed at once to the ship and consult with the other Medical Officers, who arrange for the patient's removal to the City infectious Diseases Hospital if the case is probably Cerebrospinal Fever. Meanwhile I investigate in order to get as many of the close contacts as possible. On account of ship life this is sometimes a matter of difficulty but it has been found that the contacts most likely to be Carriers are (1) those sitting close

close to the patient at mess (2) those sleeping beside him (3) any special friends (4) hospital attendants if he has been ill for any length of time, and patients in the same ward. It has been my practice to have the whole unit paraded and gradually dismiss those men not wanted. I have found the New Zealanders and Australians particularly good at volunteering necessary information about themselves and at coming forward. Nasopharyngeal swabs are taken of all the close contacts, who are segregated and kept isolated on the ship until a report is obtained. Those men in whose nasopharynges Meningococci are found are removed to the City Hospital as Carriers, and are kept there until at least two negative swabs have been obtained.

In some cases patients have been lumbar punctured on the ship, but usually the cerebro-spinal fluid is sent to the Laboratory from the City Hospital by Dr. Jasper Anderson, Medical Officer of Health, City of Cape Town who has charge of all the patients for treatment.

Owing to the belief held by some that there is a catarrhal stage in the disease, in many instances the throats of everyone on board have been inspected and swabs taken from those with acutely inflamed throats, but I cannot say that this has given any increase in the positive results. This procedure has however, rendered it possible for me to compare the results of the swabs from contacts and non-contacts. On many ships throat painting and sprays have been

been vigorously used by the Medical Officers.

The swabs are brought by motor-car to the Laboratory in fifteen to twenty minutes and immediately plated.

2. CASES OF CEREBROSPINAL FEVER.

During the period comprised, fifty-five cases of acute Cerebrospinal Fever were treated at Cape Town. In this number are NOT included (i) ten cases at one time suspicious who did not develop the disease (ii) three convalescent cases. There were also seven cases of Meningitis other than Meningococcal, five being caused by the Streptococcus and two by the Tubercle Bacillus.

The cases of Cerebrospinal fever occurring on Transports dealt with before they reached Cape Town numbered seventy-five: these were put ashore at some other port or died at sea, and where possible, contacts of these cases were examined in Cape Town.

Of the fifty-five cases treated at Cape Town fifty-one were military and four civil. They occurred as follows:-

		Military cases at Cape Town.	Total Mil. Cases on Trans-ports at & before CAPE TOWN.	Civil Cases at CAPE TOWN.
1916,	June....	11	11	-
	July ...	3.	13.	-
	August..	7.	12.	-
	September	5.	10.	-
	October..	3.	4.	-
	November.	7.	21.	-
	December.	2.	14.	-
1917	January...	2.	5.	-
	February..	-	6.	-
	March. ...	-	5.	1.
	April.....	-	-	1.
	May	1.	4.	-
	June.....	1.	2.	-.
	July.....	8.	15.	-.
	August.....	1.	4.	-.
	September..	-	-	1.
	October....	-	-	1.
	TOTAL	51.	126.	4.

It will be seen that no well marked incidence curve can be made. Enquiry as to the weather on the voyage always elicited the fact that when there were many cases the weather had been bad and the men therefore more confined below deck. Further, it appeared that the disease was more likely to develop if there were other sickness on board with consequent crowding of the Hospitals etc.

3. EXAMINATION OF CEREBROSPINAL FLUID.

The C.S. fluid was submitted for examination from 35 patients with a definite clinical diagnosis of C.S. Fever. In some cases several specimens were examined from the same patient (see below).

In 32 cases gram negative diplococci microscopically indistinguishable from Meningococci were

were found and in 28 cases Cultures of Meningococci were obtained.

In acute cases the fluids were usually distinctly turbid, often with masses of pus cells, and less turbid in milder cases.

A clear fluid is found (a) in the very early (Septicaemic) stages of an acute case (b) in slight cases (c) in cases which have become chronic.

The following table shows (a) the macroscopic characters of the fluid on primary puncture (b) whether a culture was obtained and where possible the type (c) whether meningococci were present in the Nasopharynx and where possible the type. (d) the number of specimens of C.S. Fluid examined with positive results (e) Result of disease.

NO.	NAME	DATE	SHIP.	(a)	(b)	(c)	(d)	(e)
1	El	12/6	Bel	Turbid	Pos. Type not tested.	Not examined.	1	Died
2.	Dl	18/6	Asl	Clear	No growth.	N.E.	0	died early in disease.
3.	Hu	23/8	Orl	Turbid	Type C.	Type C.	2	Died.
4.	Ll	29/8	Mil	Clear	No Growth	No Growth	0	Recovery
5.	Ml	29/8	Mil	Faintly Turbid.	Pos. Type not tested.	No Growth	1	Recovery
6.	Sl	29/8	Mil	Faintly	Pos. Type not tested.	Pos. Type not tested	1	Recovery

NO.	NAME.	DATE.	SHIP.	(a)	(b)	(c)	(d)	(e)
7.	M2	29/8	Mil	Faintly Turbid.	No growth.	Pos. type not tested.	0	Re-covery
8.	G1	18/9	Bo2	Turbid.	Type A.	Type A.	1	Died.
9.	C1	18/9	Bo2	Turbid.	Type A.	Type A.	1	Re-covery
10.	W1	18/9	Bo2	Turbid	Type A.	Type A.	3	Died.
11.	L2	3/10	P.S1	Faintly Turbid.	Type B.	Type B.	1	Recov-ery.
12.	A1	11/10	Ma2	Faintly Turbid.	Type B.	Type B.	2	Recov-ery.
13.	G1	23/10	Nel	Turbid	Type C.	Type C.	2	Died.
14.	A2	14/11	Bo3	Clear	Type C	Type C.	1	Recov-ery.
15.	L2	17/11	As1	Turbid	Type B.	not ex-amined.	1	"
16.	E2	17/11	As1	Faintly turbid.	Type B.	Type B.	2.	"
17.	M3	17/11	P.M1	Turbid	Type C.	Type C.	1	"
18.	G2	25/11	Bo4	Turbid	" C-A	" C-A	1	Died.
19.	W2	4/11	Bo5	Turbid	Type B.	Type B.	2	Recov-ery.
20.	K1	11/12	S1	Turbid	Type B.	Type B.	2	Recov-ery.
21.	M4	12/1	Me3	Turbid	Type A	Type A.	2	Died.
22.	B1	12/1	Me3	Turbid	Type A.	Type A.	7	Died.
23.	B2	6/3	Wil	Clear	No growth	No growth	0	Recov-ery.
24.	K2	22/3	Civil	Turbid	Type C.	not exam-ined.	1	Died
25.	C2	20/4	Civil	Turbid	Type C	not exam-ined.	3	Died.
26.	N84	10/5	Bo4	Blood-stained.	Type C.	" "	1	Died.
27.	W3	23/6	Sh1	Clear	No growth	" "	0	Recov-ery.

NO.	NAME.	DATE.	SHIP.	(a)	(b)	(c)	(d)	(e)
28.	M5	20/7	Hol	Clear	Pos Type not tested	Pos Type not tested.	1	Recovery.
29.	W4	21/7	Hol	Clear	"	"	1	"
30.	M6	25/7	Hol	Turbid	no growth	no growth	0	"
31.	S2	27/7	Hol	Turbid	Type B.	Type B.	2	"
32.	K3	14/9	Civil	Turbid	Type D.	Type D.	2	"
33.	P1	2/10	Civil	Turbid	Type C.	not examined.	1	Died.
34.	P2	2/10	C1	Clear	No growth	Pos. Type not tested.	0	Recovery
35.	T.	14/10	O1	Turbid	Type B.	Type B.	1	Died.

Thus from twenty fluids which were turbid, cultures of Meningococci were obtained in 19 cases: from seven faintly turbid fluids (including No. 26), in six cases; from eight clear fluids in three cases.

Twenty-one out of twenty-seven cases in which the C.S. fluid and nasopharynx were examined were positive in both. In addition to the above there were fifteen other cases in which the nasopharynx only was examined, of which fourteen were positive.

In every case in which Nasopharyngeal and Spinal Meningococci were obtained the organisms proved to be of the same type. Where more than one case occurred on the same ship, the type found present was the same for all the cases on that ship.

Two cases in which the Meningococcus was obtained P.M. are not included; nor is included a case complicated by double otitis media in which the

the organism was obtained at operation (See below).

These results are shown as follows:-

	No. of Cases.	Culture obtained	Percentage
C.S. Fluids examined	35	28	80.00
Cases with turbid C.S. fluid	20	19	95.00
" " Faintly " " "	7	6	85.71
" " Clear C.S. fluid	8	3	37.50
" in which nasopharynx was examined	41	35	85.36
" in which both C.S. fluid and nasopharynx were examined	27	21 from both C.S.F. & Nasophar.	77.77

The following Table shows the numbers of the different Types in 23 cerebrospinal strains tested:-

	No.	Recovered.	Died	Percentage Mortality.
Type A	5	1	4	80%
Type B	8	7	1	12.5%
Type C	9	2	7	77.8%
Type D	1	1	-	-
Total	23	11	12	52.2%

It will be seen that Type B Coccus was much less toxic than either Type A or C. The finding of Type B is therefore probably of good prognostic value.

Cultures of Meningococci were obtained on 1 occasion from C.S. fluids from 17 patients.
" " " were obtained on 2 occasions from C.S. fluids from 9 patients.
" " " were obtained on 3 occasions from C.S. fluids from 2 patients.
" " " were obtained on 7 occasions from C.S. fluids from 1 patient.

(Further reference is made under Miscellaneous facts of interest ii).

4. BACTERIOLOGICAL EXAMINATION OF CONTACTS AND NON-CONTACTS.

In order to obtain uniformity of results as far as possible, the selection of the contacts as already described and the taking of nasopharyngeal swabs were performed by myself. Cultures on human-blood-nasgar plates were made by touching the nasgar with the swab over a very small area and spreading the blood with a bent capillary pipette. Experiments showed that if the swabs were sufficiently saturated with nasopharyngeal mucus to prevent drying, the meningococcus if present would remain alive in this climate for at least 2 hours. As a rule, however, cultures were made within 30 minutes after the swabs were taken in order to be upon the safe side. Great care was taken to avoid contamination of the swab by salivary organisms of the mouth, but it was found to be perfectly practicable to use an ordinary diphtheria swab with careful technique.

Details of the methods of examination are given below.

The results obtained are expressed in the following table:-

	No. of Contacts.	No. of Contact-Carriers.	Percentage of Contact-Carriers.
Contacts swabbed on ships ...	844	74	8.74%
Contacts of Civil cases ..	77	7	9.09%
TOTAL CONTACTS	921	81	8·79%

	No. of non-Contacts.	No. of non-Contact Carriers.	Percentage of non-Contact Carriers.
Non-Contacts swabbed on ships	310	6	1.93%
Non-contacts other than on ships ...	141	3	2.12%
TOTAL NON-CONTACTS	451	9	1.99%

Total Contacts and non-contacts swabbed 1,372.

The carriers were further examined at frequent intervals and remained in Isolation till two consecutive nasopharyngeal swabs proved negative.

The total number of days in Hospital of 82 carriers was 3049 days which gives the average time per Carrier of 37 days.

Three of these Carriers remained positive for 120, 127 and 174 days respectively. Excluding these, the average time in hospital per carrier was 33 days.

These were made up as follows:-

TIME	NO. OF CARRIERS.
10 to 14 days	6
Under 3 weeks	23.
Under 4 weeks	16.
Under 5 weeks	12.
Under 7 weeks	5.
Under 9 weeks	7.
Under10 weeks	7.
Under12 weeks	6.

Expressed generally, the Carriers usually took three weeks to a month to be completely free. The Carriers were under the care of Dr. Jasper Anderson M.O.H. for City of Cape Town, for treatment and were kept in the open air as much as possible. Various kinds of sprays, paints and gargles were employed. I am informed by Dr. Anderson that the large amount of open air made possible in this country by the climate was one of the most important therapeutic factors. When the steam-spray method of inhalation was first described by Col. M.H. Gordon the question of its introduction was discussed here. As it was not possible to get a complete apparatus local efforts were necessary. I succeeded in getting a spraying-nozzle manufactured and connected this to the exhaust jet of an autoclave with pressure tubing and to a reservoir of chloramine (2%) or Zinc Sulphate (1%). A small room in the Laboratory was set apart as an inhalation chamber. The supply of Chloramine available at that time in Cape Town was exhausted in two days and thereafter Zinc Sulphate was employed. The latter has the advantage of being less irritating to the eyes etc. than Chloramine. Three patients received Spray treatment:-

(1) One patient Ke who after recovering from C.S. fever continued to have a practically pure culture of Meningococci present in the nasopharynx for 5 months. (This organism was of special interest as it did not belong to any of the known Types. See below).

(2) Two Carriers of two weeks duration before spraying. Nasopharyngeal swabs were taken before and after spraying and plated on blood nasgar at once. The results are as follows:-

	Ke		BAILEY		JONES.	
	Before	after	Before	after	Before	After
February 6th	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
February 8th	Pos.	Neg.	N.E.	N.E.	N.E.	N.E.
February 12th	Pos.	Neg.	Pos.	Neg.	Pos.	Pos.
February 13th	Neg.	Neg.	Neg.	Neg.	Pos.	Pos.
February 15th	N.E.	N.E.	Pos.	Neg.	Pos.	Neg.
February 19th	Pos.	Neg.	Pos.	Pos.	Pos.	Pos.
February 20th	N.E.	N.E.	Pos.	Neg.	Pos.	Pos.
February 23rd	N.E.	N.E.	Neg.	Neg.	Pos.	Pos.
February 25th	Pos.	Neg.	Neg.	Neg.	Pos.	Neg.
March 1st	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
March 5th	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
March 8th	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

N.E. = Not examined.

It is realised that the possible presence of Zinc Sulphate on the swab taken after inhalation may influence the growth. This would show, however, that the antiseptic had reached the area where it was required.

The result was specially gratifying in the case of Ke who had been, as already remarked, a carrier for 5 months. The effect of Zinc Sulphate was not so much to reduce the number of organisms in the naso-pharynx, but to favour the growth of the hardier strains at the expense of the more fragile and more pathogenic ones. In the case of Ke, the meningo-cocci were gradually replaced by a pure culture of Staphylococcus Aureus.

This 1% Zinc Sulphate Spray was also tried as a treatment for acute Catarrhal conditions but did not prove a success.

5. METHODS FOR IDENTIFICATION OF MENINGOCOCCUS.

At the time when these observations were first begun there were marked differences of opinion amongst bacteriologists what tests should be used to determine whether certain meningococcus-like organisms were or were not true meningococci. This was a matter of importance as it was necessary to do everything possible to minimise the outbreaks of cerebrospinal fever on troopships and to prevent if possible the appearance of the disease ashore at Cape Town. The latter exercised the minds of the Public Health Authorities particularly because there had been no cases amongst the civilians for some years. The problem was further complicated by the fact that it was impossible to enforce strict quarantine owing to war-exigencies.

It was left for me to devise means of detecting the Carriers liable to spread the disease. Examination of gram-negative diplococci in direct smears made from nasopharyngeal swabs showed these to be so numerous and varied as to render such an examination of little value. Attention had therefore to be turned to Cultural methods.

At that time the following tests were recommended:-

(a) Cultural appearance.

(b) Microscopic appearance.
(c) Failure to grow at 23° C.
(d) Fermentation reactions
and by some (e) Agglutination reactions.

(a) & (b) As a result of the work of Weichselbaum, (1) Von Lingelsheim (2) Flexner (3) Gordon (4) Elser and Huntoon (5) Sophian (6) and others the cultural and microscopical appearances of true meningococci had been accurately determined.

(c) Gordon had shown the value of differentiation by testing whether the organism could grow at 23°C.

(d) The fact that the Meningococcus fermented glucose and maltose and did not ferment saccharose, laevulose, mannose, lactose etc. had been established by Dunn & Gordon (7) and V. Lingelsheim (8). Minor differences were pointed out by Elser and Huntson (5). These workers further investigated M. Catarrhalis and other gram-negative cocci, descriptions of the chromogenic group and M. pharyngis siccus being given by V. Lingelsheim. Though it was thus possible to distinguish the majority of the gram negative diplococci found in the nasopharynx from true meningococci, when the evidence from these tests was taken as a whole, there remained a certain number of organisms whose nature was doubtful.

FIRST SEROLOGICAL EXPERIMENTS.

(e) I naturally turned to the agglutination test in the hope that it would prove more definite. I therefore tested the sera of 5 cases of C.S. fever

fever with emulsions of three strains of meningococci. The reactions were performed microscopically with the sera in dilutions of 1 in 40 and 1 in 80, readings being taken up to 30 minutes. Controls of normal serum and normal saline were made. It was found that in these dilutions the sera of two patients agglutinated emulsions of two cocci but did not agglutinate the third coccus. The sera from remaining three patients did not agglutinate the first two cocci but did agglutinate the third coccus. It was therefore decided to prepare immune sera with these organisms and with meningococcus - like organisms recovered from the nasopharynges of carriers.

In the meantime, agglutination tests were performed with therapeutic antimeningococcal sera. It was obvious that if a large number of doubtful strains required to be tested at one time the microscopic method would be inadequate. A macroscopic method was therefore adopted. Dilutions of serum were made and an equal proportion of emulsion of the coccus to be tested were added so that the final dilutions were 1 in 50, 100, 150, 200, 300, 400, 600, 800 and 1600. Controls of Normal serum and Normal Saline were set up, the emulsions of coccus were standardised by opacity to contain approximately 10 mgm of moist growth per c.c. Comparative tests at 37°C and 55°C were done.

The results of tests with Therapeutic Antimeningococcal sera were indefinite and unsatisfactory.

unsatisfactory. I had therefore to wait till my own antisera were ready.

(b) LITERATURE ON AGGLUTINATION.

The literature on the subject showed that the problem was somewhat complex. In 1906 Von Lingelsheim (8) found that all his 63 strains of meningococci agglutinated with sera prepared from two of them but left the question of whether certain strains from the nasopharynx of healthy persons should be regarded as pseudo-meningococci for further research. The work of Kutscher(9) Hubener and Kutscher (10) Eberle(11) and Arkwright (12) showed, however, that the problem was complex. Elser and Huntoon (5) described "agglutinable" and "inagglutinable" strains and Mayer (13) described as" pseudo-meningococci" throat strains which agreed with his standrad strains in all respects except agglutination. Friese and Muller (14) and Lieberknecht (15) did a large number of comparative tests at 37° and 55°C. and found that on the whole 55° C was the better. (Re temperature see Miscellaneous facts of Interest see below).

In 1909 Dopter (16) described certain organisms differing serologically from meningococci as "para-meningococci." He considered them nearly allied and advised that persons in whose throats they were found should be regarded as Carriers of the meningococcus. Mayer, Waldmann, Furst and Gruber (17) found 1.73% of Carriers among 9,111 healthy and concluded that the

the meningococcus must be regarded for practical purposes as ubiquitous and that bacteriological examination of throats was impracticable as a useful aid to prophylaxis in time of epidemic. Further work on agglutination tests was reported by Sachs-Muke (18), and on complement fixation tests by Arkwright (19) in 1911.

In 1912 an advance was made by Dopter (20) who reported 12 cases of clinically typical C.S. fever due to para-meningococci in which anti-para-meningococcal serum proved successful. In 1914 Darre and Dumas (21) obtained parameningococci serologically different from those of Dopter, and in the same year Dopter and Pauron (22) differentiated three groups the L, B and J varieties of parameningococci. Much of this was unknown to me at the time, but in any case the general feeling was that the agglutination test was unreliable as a means of identifying the Meningococcus. I was therefore keen to see if the difficulties could not be overcome.

(c) PREPARATION OF ANTIMENINGOCOCCAL SERA.

For this purpose rabbits were used, all injections being made intravenously into the marginal vein of the ear.

A. Four rabbits each received living emulsions in .85% Saline of 20,000 million organisms of strains Hul Sp, Gl Sp and Wl Sp (All from C.S.F.) and An. (Nasophar.). All animals were very ill, Hul Sp and Wl Sp dying within 3 days. Post mortem did not show anything definite.

B. The dose given to the surviving Gl Sp and

and An. (Nasoph) rabbits was reduced to 10,000 million.

Inoculation of 4 other rabbits was commenced with the same dose viz. Hul Sp, Wl Sp, An.Sp. and Ho Np. All animals were at first ill and Ho Np. died. The other animals received 7 further doses at intervals of 6 to 8 days. The sera were found 10 days later to have a titre of approximately 1 in 1600 for the homologous coccus.

At this point one animal Hul Sp became very ill, and showed clinical appearances typical of Meningitis. As this is of great interest further details are given below on page 52. The titre of serum obtained was approximately 1-1600.

Two other rabbits Gl Sp and Wl Sp received 7 more inoculations of 10,000 million each (making a total of 15 injections) but without the production of symptoms. The titre of the sera of these rabbits remained at approximately 1-1600 during this period.

C. A series of rabbits was inoculated intravenously with strains W2 Sp., Coh. Sp iii and Ken. Sp 1. A dose of 2000 million killed cocci in 1 c.c. of .85% Saline was given (a) in the morning (b) in the evening (c) the following morning. With an interval of two days, three similar doses of living cocci were given, and again with the same interval three doses of 3000 or 4000 million. After 8 days serum with a titre of 1 in 800 to 1 in 1600 was obtained. None of the animals became ill.

The results of Agglutination tests are given later.

METHODS OF IDENTIFICATION.

As a basis for study of Meningococci, especially with a view to identifying nasopharyngeal strains, I made a close study of Gram-negative diplococci recovered from the Cerebrospinal fluid of cases typical of Cerebrospinal fever. The examination included (1) direct microscopical examination of centrifugalised deposit, (2) the study of macroscopical and microscopical cultural characters, (3) fermentation tests, (4) agglutination and (5) absorption tests.

After thoroughly studying Strains Hu Sp, Gl Sp and Wl Sp, I was in a position to detect probable nasopharyngeal meningococci. There is no need to refer further to (1) and (2) above.

(d) FERMENTATION TESTS.

These were done with known meningococci and with gram-negative diplococci of doubtful nature. At first I used the "sugars" prepared for routine purposes in the Laboratory (i.e. Veal broth with 1% Peptone and 1% Sugar with specially prepared blue litmus to a deep blue) with the addition of 6 drops of sterile horse-serum to 1 c.c. of medium. (The addition of sterile ascitic fluid was also tried but was inferior to horse-serum.) The tests were conconveniently carried out in tubes of 4 ins. by $\frac{5}{8}$ in. The reactions, however, were only slowly produced, and the presence of the smallest contamination with Streptococci vitiated the results. It was further necessary to check the growth by Subculture. I

I therefore adopted a solid medium of litmus-starch--nasgar with 1% of the test "sugar" added. A reaction was usually obtained in 12 to 24 hours with streak cultures and the method avoided the disadvantages of the fluid media. Contaminations were easily detected.

The organisms most likely to be confused with meningococci were unfortunately those in which the fermentation reactions were the most similar. For a short time M. Pharyngis Siccus, M. Flavus 1 and 11 and M. Catarrhalis were tested but those causing real difficulty were M. Flavus 111 and possible "pseudo-meningococci". In young colonies of M. Flavus 111 the pigment is often very slight, and the fermentation of laevulose is thus of value. It is obviously necessary to be sure of the purity of the "Sugars" and with specimens of absolutely pure laevulose, meningococci (i.e. from the C.S. fluid of cases of C.S. fever) did not show production of acid on Solid Laevulose Media. An acid reaction was frequently followed by an alkaline reaction, especially with laevulose, in cases of M. Flavus 111, but this also occurred with Meningococci and M. Flavus 111 in Glucose and Maltose to a less extent. This does not seriously affect the test.

T A B L E.
217 Sugar reactions.

No.of Strains	Glu-cose	Mal-tose	Laevu-lose.	Saccha-rose.	Lac-toss	Man-nite.	REMARKS.
16	-	-	-	-	-	-	All M. Catarr. except one viz. Gl. -C.S.F. Meningo. (see below).
11	@@	@@	@@	@@	-	-	All M. Flavus 1 or 11 or M.P. Siccus.
96	@@	@@	@@	-	-	-	All M. Flavus III.
90	@@	@@	-	-	-	-	32 C.S.F. Meningococci, 55 Naso-phar. ? Meningo & 3 M. Flavus III.
4	-	@@	-	-	-	-	1 C.S.F. Meningo-coccus & 3 Naso-pharyngeal meningococci.

217 gram-negative diplococci were tested regarding their action on Glucose, Maltose, Laevulose, Saccharose, Lactose and Mannite, Of these, (a) 125 were culturally :- 15 M. Catarrhalis, 11 M. Flavus 1,11, and M. P. Siccus, 99 M. Flavus III.

(b) 92 were culturally meningococci, made up of 34 strains from C.S. Fluid and 58 from Nasopharynges.

Re (a) All 15 M. Catarrhalis failed to ferment any of the above "Sugars". The 11 strains of M. Flavus 1, and 11 and M.P. Siccus fermented Glucose, Maltose, Laevulose and Saccharose. 96 of the 99 strains of M. Flavus 11 fermented Glucose, Maltose and Laevulose only, but 3 strains with undoubted Flavus pigment did not ferment Laevulose and only fermented Glucose and Maltose.

Re (b) 32 of the 34 Meningococci from C.S. fluids and

and 55 of the 58 Nasopharyngeal meningococci fermented Glucose and Maltose only. Of these, 17 produced a stronger reaction in Maltose than in Glucose. And in 7 the reaction in Glucose was stronger than in Maltose. One cerebrospinal strain and 3 Nasopharyngeal strains fermented Maltose only.

It was found that the strains which fermented Maltose more than Glucose belonged chiefly to Types A & C as did the strains which fermented Maltose only, but it was impossible to make a definite classification on this basis.

In one instance a Cerebrospinal strain G1. failed to ferment any of the six "Sugars". This was an undoubted meningococcus from a case which proved fatal, and it was found to belong to Type A (Gordon's Type III) by agglutination and absorption tests, and by agglutinogenic capacity. The fermentation reactions were tested frequently at intervals and in no instance was any sugar fermented.

Had this organism been recovered from the nasopharynx and subjected only to microscopical, Cultural and fermentative tests its identity as a meningococcus would probably have been missed.

Though the fermentation reactions were of great assistance in identifying doubtful organisms, in many cases investigation required to be continued and repeated in order to obtain satisfactory results. On the other hand as accurate a diagnosis as possible was required with as little delay as possible. I therefore felt the great need for a satisfactory agglutination test as confirmatory evidence.

(e) AGGLUTINATION TESTS.

As has been stated, experiments with therapeutic antimeningococcal sera were indefinite and unsatisfactory.

As soon as agglutinating sera could be obtained from rabbits as described above, tests were made to establish a standard method.

(a) Emulsions were made from slopes of Starch-Nasgar and heated to 65°C for ½ hour. Results with heated emulsions were found to be more uniform than with unheated emulsions.

(b) A measured sample was withdrawn and diluted with .85% Saline to 1000 million per c.c. estimated by opacity. The bulk was then titrated from this calculation to 10,000 million per c.c. Phenol was added to make .5 per cent present. (Later, the method described by Gordon of diluting .1 c.c. with tap-water till it is just turbid was adopted. This end point is taken to represent 100 million per c.c. and the bulk was titrated accordingly.) A portion of the bulk emulsion is diluted to 2000 million per c.c. with .85% Saline and .5% Phenol, and the remainder kept.

(c) It was found by comparative experiments that it was possible to make readings with cocci in final dilutions of 500 million, 1,000 million, 2000 million and 4000 million per c.c., other conditions being the same, and all the organisms in each test being at the same dilution. With 500 million the emulsions were too thin, and a final dilution of 1000 million per c.c. was selected as being the most dilute emulsion with which good readings could be made.

(d) It was found that incubation at 55°C overnight gave better results than at 37°C. This could be expected from consideration of the nature of the test. (Further remarks on this are made below.)

(See page 55 V)

METHOD FOR AGGLUTINATION.

(a) Dilution of Serum.

Large test-tubes containing 1-25, 1-50, 1-100, 1-200, 1-400 dilutions of each test serum are arranged in rows of five. Dilutions are made with freshly prepared .85% Saline in sufficient quantity to allow .5 c.c. of each dilution for each coccus to be tested and controls. One test-tube with 1-25 Normal rabbit-serum and one with .85% Saline are added.

For each test serum a plasticine tray is prepared by placing small tubes 2 by five-sixteenths inches in as many horizontal rows of five as there are cocci to be tested including controls. On one tray the number in each horizontal row is increased to seven to provide for one Normal serum control and one Normal Saline control of each coccus . Two extra tubes are placed on each of the other trays to provide for Normal Serum and Saline Controls for the homologous control coccus. Into each vertical row of these small tubes .5 c.c. of the corresponding dilution of serum or normal saline is now placed. A fresh clean sterilized pipette is required for each serum. Pipettes used are .5 c.c. and 1 c.c. graduated to .05 c.c. A rubber nipple is used.

(b) .5 c.c. of a 2000 million emulsion of each coccus to be tested is then added to the tubes in one of the rows of each plasticine tray i.e. to a series of dilutions of each test serum, to 1-25 Normal serum and .5 c.c. of .85% Saline. .5 c.c. of a 2000 million per c.c. emulsion of the homologous coccus is added to a series of dilutions of the corresponding serum as a control, to 1-25 Normal serum and to .5 c.c. of .85% saline. A fresh clean sterilized pipette is used for each coccus. The tubes are well shaken and are then either covered by a "blanket" of plasticine or tightly plugged with cotton wool. They are then placed in the 55°C incubator over-night.

READING RESULTS.

The tubes are examined in the following order:-

(1) Normal-rabbit-serum control coccus, and normal saline control coccus.

(2) Control homologous coccus with corresponding serum 1-50 to 1-800.

(3) Normal serum and Normal Saline controls of first test coccus.

(4) First test coccus with dilutions of agglutinating serum 1-50 to 1-800.

and so on with each test coccus, the normal serum and saline controls being examined first. It is adviseable for two people to work together when possible, one recording results as each tube is examined. In order that the readings may be impartial, numbers are used

used and a key of the cocci concerned is made before the coccal emulsions are added. This key is consulted only when the results have been recorded. The signs used for different degrees of agglutination are given below. Unless the controls are satisfactory no conclusions can be made.

In order that the tests may be comparative it is necessary that the titre of each serum is approximately the same. This is accomplished by dilution of the stronger sera in bulk with .5% Phenol in .85% Saline so that when used in a test they give complete agglutination at 1-400 and a partial reaction at 1-800. When this is once accurately done the same dilutions can be used throughout subsequent tests with all the sera.

(f) RESULTS OF AGGLUTINATION TESTS.

The first tests were performed with three sera prepared from cerebrospinal strains Gl Sp. Hu Sp Wl Sp. which were the first three strains recovered by me.

TABLE 1.

COCCUS.	N.S.	Serum G1 Sp.					Serum Hu1 Sp.					Serum W1 Sp.					Normal Saline.
	1-50	1-50	100	200	400	800	1-50	100	200	400	800	1-50	100	200	400	800	Normal
G1 Sp.	-	@@@	@@@	@@	@@	@@	@@	@@	@	-	-	@@@	@@	@@	@@	@@	-
Hu 1 Sp	-	@@	@@	@	-	-	@@@	@@@	@@	@@	@	@@	@@	@	-	-	-
W1 Sp	-	@@@	@@	@@	@@	@@	@@	@@	@	-	-	@@@	@@@	@@	@@	@@	-
W2 Sp.	-	@	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavus.	@@	@@@	@@@	@@	@@	@	@@@	@@@	@@	@@	@	@@@	@@@	@@	@@	@	-

In this and subsequent tables, @@@ indicates complete agglutination with clear supernatant fluid and a very copious deposit, @@ indicates complete agglutination with clear fluid but a smaller deposit and @ indicates definite agglutination with some deposit but with slight turbidity remaining in the fluid. The nature of the deposit will be referred to later.

As Coccus W2 Sp. did not agglutinate with any of the above sera, an antiserum was prepared with it from a rabbit, and used with one or more of the other sera for diagnostic purposes.

In Table 2 the results of agglutination of 24 Meningococci with sera W1 Sp and W2 Sp. are given. The organisms tested were taken at random and during the tests were represented by a number.

The results are noted in the table without rearrangement but numbers replaced by recognisable signs. Spinal and Nasopharyngeal strains are indicated by Sp. and np. respectively. Roman numerals indicate the number of particular strain from patient e.g. B1 Sp. vii is the 7th Strain obtained from the 7th specimen of C.S. Fluid from pateint B1.

SERUM W1 Sp.

It will be seen that (1) G1 Sp., B1 Sp. vii and the homologous coccus W1 Sp. completely agglutinate at 1-400 and Mu1 Sp. at 1-200 with serum W1 Sp. and practically not at all with serum W2 Sp.

(2) Strains Gill. Sp., N 84 Sp., Hu1 Sp., Dev. Np iv., Coh. Sp lll, Greg. Sp., give partial agglutination with serum W1 Sp. but not with serum W2 Sp.

(3) Strains Ho Sp., W2 Np., W2 Sp., Clen Np. Dyb. Np., C. Mor. Np., Key. Sp. ll give no agglutination with serum W1 Sp. but are strongly positive with serum W2 Sp.

(4) The five strains from patient Ke. Viz. Ke Sp. 1 & ll and Ke Np. 1,ll and lll all fail to agglutinate with either serum.

TABLE 2.

COCCUS	Agglutination Serum W1 Sp. Normal Serum. 1-50	1-50	100	200	400	800	Agglutination Serum W2 Sp. 1-50	100	200	400	800	Na.Cl Control
Ho.Sp.	-	-	-	-	-	-	@@@	@@@	@@	@@	@	-
W2 Np.	-	-	-	-	-	-	@@@	@@@	@@	@@	@	-
G1 Sp.	-	@@@	@@@	@@	@@	@	@	-	-	-	-	-
B1 Sp. vii	-	@@@	@@@	@@	@@	@	@	-	-	-	-	-
Gill Sp.	-	@@	@@	@@	@	-	-	-	-	-	-	-
Ke Np iii	-	@	-	-	-	-	@@	@	-	-	-	-
Ke Np i.	-	-	-	-	-	-	-	-	-	-	-	-
Ke Sp ii	-	@	-	-	-	-	@	-	-	-	-	-
Mul.Sp.	-	@@	@@	@@	@	-	-	-	-	-	-	-
W2 Sp.	-	-	-	-	-	-	@@@	@@@	@@	@@	@	-
W1 Sp.	-	@@@	@@@	@@	@@	@	-	-	-	-	-	-
Ke Sp i.	-	-	-	-	-	-	@	-	-	-	-	-
Clem. Np.	-	-	-	-	-	-	@@@	@@@	@@	@@	@	-
Dyb. Np.	-	@	-	-	-	-	@@@	@@	@@	@@	@	-
C. Mor. Np.	-	@	-	-	-	-	@@@	@@	@@	@@	@	-
Bro Np. i.	-	@@	@	-	-	-	@@	@	-	-	-	-
N 84 Sp.	-	@@	@@	@	-	-	@	-	-	-	-	-
Hul Sp.	-	@@	@@	@	-	-	@	-	-	-	-	-
Coh. Sp. iii	-	@@	@	-	-	-	@	-	-	-	-	-
Dev. Np.iv	-	@@	@@	@	-	-	@	-	-	-	-	-
Ke Np. ii	-	-	-	-	-	-	-	-	-	-	-	-
Greg. Sp.	-	@@	@@	@	-	-	@	-	-	-	-	-
Bro. Np. iv	-	@@	@@	@@	@	-	@@	@@	@	-	-	-
Key.Sp.ii.	-	-	-	-	-	-	@@@	@@	@@	@	-	-

(5) Bro. Np 1 & iv give approximately equal reactions with both sera respectively.

SERUM W2 Sp.

Taking the serum W2 Sp. table as a whole, 6 cocci give complete agglutination at 1 in 400 and 1 coccus at 1 in 200, whilst 14 cocci give no more than a partial reaction at 1-50, 2 cocci full agglutination at 1-50 and 1 coccus at 1-100.

From these tables it is clear that:-

(1) G1 Sp., B1 Sp. vii, and W1 Sp. are serologically nearly related and distinct from the rest,

(2) 7 cocci are able to be serologically distinguished by serum W2 Sp. from the remainder.

(3) 9 other cocci show relationship chiefly to W1 Sp.

(4) 5 cocci from patient Ke. show no relationship to either serum.

From the last named two groups two cocci were selected viz. Coh Sp iii and Ken. Sp. 1 and antisera were prepared from these.

TABLE 3.

COCCUS	Normal Serum. 1-50	Agglutinating Serum Coh. Sp. 111. 1-50	100	200	400	800	Agglutinating Serum Ken. Sp. 1. 1-50	100	200	400	800	Na.Cl Control.
Ho Sp.	-	-	-	-	-	-	-	-	-	-	-	-
W2 Np.	-	-	-	-	-	-	-	-	-	-	-	-
G1 Sp.	-	@@	@@	@@	@	-	-	-	-	-	-	-
B1 Sp. vii	-	@@	@@	@	-	-	-	-	-	-	-	-
Gill.Sp.	-	@@@	@@	@@	@@	@	-	-	-	-	-	-
Ke Np 111	-	@	-	-	-	-	@@@	@@@	@@	@	-	-
Ke Np 1.	-	-	-	-	-	-	@@	@@	@@	@	-	-
Ke Sp 11.	-	-	-	-	-	-	@@@	@@@	@@	@@	@	-
Mul Sp.	-	-	-	-	-	-	-	-	-	-	-	-
W2 Sp.	-	@	-	-	-	-	-	-	-	-	-	-
W1 Sp.	-	-	-	-	-	-	-	-	-	-	-	-
Ke.Sp. 1	-	-	-	-	-	-	@@@	@@@	@@	@@	@	-
Clen.Np.	-	@	-	-	-	-	-	-	-	-	-	-
Dyb. Np.	-	@	-	-	-	-	-	-	-	-	-	-
C. Mor. Np.	-	@@	@@	@	-	-	-	-	-	-	-	-
Bro. Np.1.	-	-	-	-	-	-	-	-	-	-	-	-
N. 84 Sp.	-	@@@	@@	@@	@@	@	-	-	-	-	-	-
Hu 1 Sp.	-	@@	@@	@@	@@	-	-	-	-	-	-	-
Coh. Sp. 111	-	@@@	@@	@@	@@	@	-	-	-	-	-	-
Dev. Np. iv.	-	@	@	-	-	-	-	-	-	-	-	-
Ke.Np. ii	-	@	@	-	-	-	@@@	@@	@@	@	-	-
Greg.Sp.	-	@@@	@@	@@	@@	@	-	-	-	-	-	-
Bro. Np. iv	-	@	-	-	-	-	@@	@	-	-	-	-
Key Sp. ii.	-	-	-	-	-	-	-	-	-	-	-	-

With reference to Serum Coh.Sp. lll. it can be stated that complete agglutination took place with

(1) Gill Sp., N84 Sp., Greg. Sp. and homologous coccus Coh. Sp. lll at 1-400.

(2) Gl Sp. and Hul Sp. at 1-200.

(3) C. Mor. Np. at 1-100. With this slight exception no reaction took place with any of the 7 cocci which agglutinate with serum W2 Sp.

All 5 Ke. cocci are negative with Serum Coh. Sp. lll.

Reference to Serum Ken. Sp. 1. will show that Ke.Sp. 1 and 11 both completely agglutinate at 1-400 and the Ke. Sp. 1, 11, and 111 all at 1-200 whilst, apart from a reaction at 1-50 with Bro. Np. iv, none of the other cocci show any reaction with this serum. Further observations regarding the cocci from this patient are made below.

The main results are summarised in Table 4.

TABLE 4.

TITRE AT WHICH AGGLUTINATION IS COMPLETE.

Coccus.	Serum W1 Sp. Titre 1–400	Serum W2 Sp. Titre 1–400	Serum Coh.Sp. III. Titre 1–400	Serum Ken.Sp.1 Titre 1–400		
W1 Sp.	400	–	@	–	Type "A"	Types A&C.
B1 Sp.vii	400	@	100	–		
G1 Sp.	400	@	200	–		
Mul.Sp.	200	–	–	–		
Coh.Sp.iii	50	@	400	–	Type "C"	
Gill.Sp.	200	–	400	–		
N84 Sp.	100	@	400	@		
Greg.Sp.	100	@	400	–		
Hul.Sp.	100	@	200	–		
C.Mor.Np.	–	400	100	–	Type "B".	
W2 Sp.	–	400	@	–		
W2 Np.	–	400	–	–		
Ho Sp.	–	400	–	–		
Clen Np.	–	400	@	–		
Dyb. Np.	–	400	@	–		
Key Sp. II	–	200	–	–		
Ken.Sp. 1	–	@	–	400	Type "D"	
Ken.Sp. II.	–	@	–	400		
Ken. Np.1	–	–	–	200		
Ken. Np.II	–	–	@	200		
Ken.Np.III	–	50	@	200		
Bro.Np.iv.	200	100	@	50	Scrap heap.	
Bro.Np.1.	50	50	–	–		
Dev.Np.iv.	100	@	@	–		

The sign @ indicates that partial agglutination took place at a dilution of 1–50.

From the above table it will be seen that

(1) By the action of Serum W2 Sp. the cocci can be resolved into two main groups. As this was the second serum to be tried, the group agglutinating with this serum was named Type B.

(2) The first four cocci agglutinate better with serum W1 Sp. than with Serum Coh. Sp. 111 and were named Type A.

(3) The following five cocci fall in a group named Type C.

(4) There is a varying relationship shown by agglutination test between members of Types A & C but both these types are distinct from Type B.

(5) Cocci Ke. Sp. 1 and 11, and Np. 1,11, and 111 are serologically distinct from these three types.

(6) Three Np. cocci Bro. N.P. 1, and iv and Dev. Np.iv remain as a "scrap-heap", it being doubtful whether they are meningococci at all.

Up to this time I had carried out the above work independently but my attention was now drawn to the work being done upon the subject by Lt.-Col. Mervyn Gordon and his colleagues (23) and by the Medical Officers of the Local Government Board of England (24). Through the kindness of Capt. E.G. Murray R.A.M.C. who visited me here, I was enabled to send live cultures of 30 strains of meningococci to Lt.-Col. Gordon. These were grown as Stabs upon Starch-nasgar, the plugs being sealed with wax, and during the journey from South Africa were subjected to ordinary temperature. Three strains were found

found to have survived. That so many had died is not so surprising as that any had survived.

Col. Gordon reported that two strains belonged to his Type II and one to his Type IV. By the time this information was received I had unfortunately lost the strain of this last organism.

Later, I dispatched 37 killed Coccal emulsions to Col. Gordon and received the results of his examinations after the work recorded below was completed. Further reference is made below.

The questions that presented themselves were:-

(1) Will this method satisfactorily differentiate true Meningococci from other gram-negative diplococci?

(2) Is this grouping a permanent and a sound one?

(3) If reliance is put on this method will any true Meningococci be excluded?

Col. Gordon and his colleagues considered that the first two questions should be answered in the affirmative. On the other hand the Medical Officers of the Local Government Board considered that the last question should be answered by "Yes".

(g) ABSORPTION OF AGGLUTININS.

This was carried out with 45 strains of organisms which had the cultural, microscopical and fermentative characters of Meningococci and with one typical strain of M. Flavus III. (One exception already referred to, differed in fermentative characters.)

Of the Meningococci, 14 were strains from C.S. fluids of definite cases of C.S. fever, 6 were from Nasopharynges of patients with C.S. fever, and 25 were Nasopharyngeal strains from contacts of cases.

The method adopted was based on the modification of Castellani's method described by Gordon in the R.A. Medical Corps Journal Vol. XXV No. 4.

Modifications were found necessary as follows:-

(A) A single saturation at 37°C was found to be insufficient for the test coccus to remove all the agglutinins with which it would combine. Hence,

(1) the original serum dilution was made 1 in 12.5.

(2) .5 c.c. of diluted serum plus .5 c.c. of test coccal emulsion were incubated for 24 hours at 37°C.

(3) Centrifugalised for ½ hour.

(4) .5 c.c. of supernatant plus .5 c.c. of test coccal emulsion were incubated for 24 hours at 37°C.

(5) The final dilution was thus 1 in 50 of a serum with titre giving complete agglutination at 1 in 400 and partial agglutination at 1 in 800 with homologous coccus.

(6) After being again centrifugalised for ½ hour, the supernatant from each tube was diluted and tested for agglutination with the homologous coccus. Controls of test coccus and " absorbed" serum were made in every case, and were required to be negative before any conclusion could be drawn.

(B). Emulsions of 2000 million per c.c. (i.e. 1,000 million organisms in each absorption) were found to be too dilute and 8,000 million per c.c. were therefore employed for saturation. For all the actual agglutination tests, including that of supernatant with homologous cocci, emulsions of 2000 million were used.

In the agglutination tests previously recorded 24 cocci were used and the results recorded in tables in the order in which the tubes happened to come.

In the following "absorption tests" the same 4 sera were used but 46 cocci were tested, including some of those appearing inthe Tables above. The results of absorption tests with 36 of these appear in the following four tables. During the tests the numbers in the table were employed, the cocci being in no arranged order. After the results were noted the names were filled in from a key and the results arranged according to reactions as follows. The remaining 10 cocci are considered in separate tables later.

TABLE 5.

Serum "Wl Sp" Type A (Type III Gordon)

Test Coccus.		AGGLUTINATION					ABSORPTION. v. Homologous coccus.				v. Test coccus.	
		1-50	100	200	400	800	100	200	300	400	100	200
Wl Sp	2	@@@	@@@	@@	@@	@	-	-	-	-	-	-
Mul Sp.	4	@@	@@	@@	@	-	@	-	-	-	-	-
Bl Sp. vii	9	@@@	@@@	@@	@@	@	@	-	-	-	-	-
Gl Sp.	10	@@@	@@@	@@	@@	@	@	-	-	-	-	-
Tl Np.	32	@@@	@@	@@	@	-	@@	@	-	-	-	-
W2 Sp.	3	-	-	-	-	-	@@@	@@	@@	@@	-	-
W2 Np.	11	-	-	-	-	-	@@@	@@	@@	@@	-	-
Clen.Np	24	-	-	-	-	-	@@@	@@	@@	@@	-	-
Dyb.Np.	23	@	-	-	-	-	@@@	@@	@@	@@	-	-
Mor.Np	22	@	-	-	-	-	@@@	@@	@@	@@	-	-
Key.Sp.	13	-	-	-	-	-	@@@	@@	@@	@@	-	-
Sh.Np.	45	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ho.Sp.	12	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ho.Np.	34	-	-	-	-	-	@@@	@@	@	@	-	-
Mc.Np.	31	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ma.Np.	29	-	-	-	-	-	@@@	@@	@@	@	-	-
Du.Np.	27	-	-	-	-	-	@@@	@@	@@	@	-	-
Ph.Np.	30	-	-	-	-	-	@@@	@@	@@	@	-	-
Br.Np.	35	@	-	-	-	-	@@	@@	@@	@	-	-
Ch.Np.	25	-	-	-	-	-	@@	@@	@@	@	-	-
Da.Np.	41	@	-	-	-	-	@@	@@	@@	@	-	-
Coh.Sp.	18	@@	@	-	-	-	@@	@@	@@	@@	-	-
La.Np.	46	@@	@	-	-	-	@@	@@	@	-	-	-
Gr.Sp.	15	@@	@@	@	-	-	@@	@@	@	-	-	-
N84 Sp.	20	@@	@@	@	-	-	@@@	@@	@@	@	-	-
Gil.Sp.	8	@@	@@	@@	@	-	@@	@@	@	-	-	-
Ne.Np.	39	@@	@@	@@	@	-	@@	@@	@	-	-	-
Hu.Sp.	19	@@	@@	@	-	-	@@	@@	@@	@	-	-
Ca.Np.	24	@@	@	-	-	-	@	-	-	-	-	-
Br.Np.	26	@@	@@	@	-	-	@	-	-	-	-	-
Ke Sp 1.	1	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ke.Sp.2	5	@	-	-	-	-	@@@	@@	@@	@	-	-
Ke Np 1.	6	-	-	-	-	-	@@@	@@	@@	@	-	-
Ke Np 2.	16	-	-	-	-	-	@@@	@@	@@	@	-	-
Ke Np 3.	7	@	-	-	-	-	@@@	@@	@@	@@	-	-
Ke Np 4.	43	-	-	-	-	-	@@@	@@	@@	@	-	-

TABLE 5 SHOWS:-

(1) W1 Sp. "absorbs" fully the agglutinins of homologous serum.

(2) Mul Sp, B1 Sp VII, G1 Sp absorb almost completely as do also Ca Np and Br Np. lower down in the series. T1 Np does so to a slightly less degree.

(3) The group from W2 Sp down to Da Np inclusive, which do not agglutinate, do not appreciably absorb any agglutinin.

(4) Of group Coh Sp to Hu Sp, which show some agglutination, 4 cocci show slight absorption and 3 do not.

(5) The 2 Spinal and 4 Nasopharyngeal strains from same patient Ke, which do not agglutinate, show no absorption. Thus, W1 Sp down to T1 Np probably belong to the same group whilst Coh Sp down to Br Np belong to a separate but associated group. (The reason for including Ca Np and Br Np here will be seen later)

In Table 6 which follows, it will be seen that none of the cocci absorb agglutinin from Serum W2 Sp. except those from W2 Sp down to Da Np which constitute the group referred to in para (3) above. All of these cocci give "one plus" agglutination at 1 in 400 or 1 in 800. The correspondence of absorption results with those of agglutination is most striking.

La Np gives "one-plus" agglutination at 1 in 200 but does not appreciably absorb.

TABLE 6.

Serum W2 Sp. Type B. (Type 11 Gordon)

Test Coccus . No.	AGGLUTINATION					ABSORPTION. v. Homologous Coccus.				v. Test Coccus.	
	1-50	100	200	400	800	100	200	300	400	100	200
W1 Sp. 2	-	-	-	-	-	@@@	@@	@@	@@	-	-
Mul Sp. 4	-	-	-	-	-	@@@	@@	@@	@@	-	-
B1 Sp.vii 9	@	-	-	-	-	@@	@@	@@	@@	-	-
G1 Sp. 10	@	-	-	-	-	@@@	@@	@@	@@	-	-
T1 Np. 32	-	-	-	-	-	@@@	@@	@@	@@	-	-
W2 Sp. 3	@@@	@@@	@@	@@	@	-	-	-	-	-	-
W2 Np. 11	@@@	@@@	@@	@@	@	-	-	-	-	-	-
Clen.Np. 24	@@@	@@@	@@	@@	@	-	-	-	-	-	-
Dy b.Np. 23	@@@	@@	@@	@@	@	-	-	-	-	-	-
Mor. Np. 22	@@@	@@	@@	@@	@	-	-	-	-	-	-
Key .Sp. 13	@@@	@@	@@	@	-	-	-	-	-	-	-
Sh.Np. 45	@@@	@@	@@	@@	@	@	-	-	-	-	-
Ho.Sp. 12	@@@	@@@	@@	@@	@	-	-	-	-	-	-
No.Np. 34	@@@	@@	@@	@	-	-	-	-	-	-	-
Mc. Np. 31	@@@	@@	@@	@	-	-	-	-	-	-	-
Ma Np. 29	@@@	@@	@@	@	-	-	-	-	-	-	-
Du Np. 27	@@@	@@	@@	@	-	-	-	-	-	-	-
Ph Np. 30	@@@	@@	@@	@	-	-	-	-	-	-	-
Br. Np. 35	@@@	@@	@@	@	-	-	-	-	-	-	-
Ch Np. 25	@@	@@	@	-	-	@	-	-	-	-	-
Da Np. 41	@@	@@	@@	@	-	@@	@	-	-	-	-
Coh Sp. 18	@	-	-	-	-	@@@	@@	@@	@@	-	-
La Np. 46	@@	@@	@	-	-	@@@	@@	@@	@@	-	-
Gr Sp. 15	@	-	-	-	-	@@	@@	@@	@	-	-
N84 Sp. 20	@	-	-	-	-	@@@	@@	@@	@@	-	-
Gil. Sp. 8	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ne Np. 39	@	-	-	-	-	@@	@@	@@	@	-	-
Hu Sp. 19	@	-	-	-	-	@@	@@	@@	@	-	-
Ca Sp. 28	@@	@	-	-	-	@@	@@	@	-	-	-
Br Np. 26	@@	@	-	-	-	@@	@@	@@	@	-	-
Ke Sp.1. 1	@	-	-	-	-	@@@	@@	@@	@@	-	-
Ke Sp 2. 5	@	-	-	-	-	@@@	@@	@@	@@	-	-
Ke Np.1. 6	-	-	-	-	-	@@	@@	@@	@	-	-
Ke Np 2. 16	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ke Np 3. 7	@	-	-	-	-	@@@	@@	@@	@@	-	-
Ke Np 4. 43	-	-	-	-	-	@@@	@@	@@	@@	-	-

TABLE 7.

Serum Coh Sp. Type C (Type 1 Gordon)

Test Coccus	No.	AGGLUTINATION					ABSORPTION v Homologous Coccus.				ABSORPTION v Test Coccus.	
		1-50	100	200	400	800	100	200	300	400	100	200
W1 Sp.	2	-	-	-	-	-	@@@	@@	@@	@@	-	-
Mul Sp.	4	@@	@	-	-	-	@@@	@@	@@	@@	-	-
B1 Sp.vii	9	@@	@@	@	-	-	@@@	@@	@@	@@	-	-
G1 Sp.	10	@@	@@	@@	@	-	@@@	@@	@@	@@	-	-
T1 Sp.	32	@@	@	-	-	-	@@@	@@	@@	@@	-	-
W2 Sp.	3	@	-	-	-	-	@@@	@@	@@	@@	-	-
W2 Np.	11	-	-	-	-	-	@@@	@@	@@	@@	-	-
Clen Np.	24	@	-	-	-	-	@@@	@@	@@	@@	-	-
Dyb Np.	23	@	-	-	-	-	@@@	@@	@@	@@	-	-
Mor Np.	22	@@	@@	@	-	-	@@@	@@	@@	@@	-	-
Key Sp.	13	-	-	-	-	-	@@@	@@	@@	@@	-	-
Sh Np.	45	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ho Sp.	12	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ho Np.	34	-	-	-	-	-	@@@	@@	@@	@@	-	-
Mc Np.	31	-	-	-	-	-	@@	@@	@@	@	-	-
Ma Np.	29	@@	@	-	-	-	@@	@@	@@	@	-	-
Du Np.	27	-	-	-	-	-	@@	@@	@@	@@	-	-
Ph Np.	30	-	-	-	-	-	@@	@@	@@	@@	-	-
Br Np.	35	@@	@	-	-	-	@@@	@@	@@	@@	-	-
Ch Np.	25	@	-	-	-	-	@@@	@@	@@	@@	-	-
Da Np.	41	@	@	-	-	-	@@@	@@	@@	@@	-	-
Coh Sp.	18	@@@	@@	@@	@@	@	-	-	-	-	-	-
La Np	46	@@@	@@	@@	@@	@	-	-	-	-	-	-
Gr Sp.	15	@@@	@@	@@	@@	@	@	-	-	-	-	-
N84 sp.	20	@@@	@@	@@	@@	@	@	-	-	-	-	-
Gil Sp.	8	@@@	@@	@@	@@	@	@	-	-	-	-	-
Ne Np.	39	@@@	@@	@@	@@	@	-	-	-	-	-	-
Hu Sp.	19	@@	@@	@@	@	-	@@	@	-	-	-	-
Ca Sp.	28	@@@	@@	@@	@@	@	-	-	-	-	-	-
Br Np.	26	@@@	@@	@@	@@	@	-	-	-	-	-	-
Ke Sp1.	1	-	-	-	-	-	@@	@@	@@	@@	-	-
Ke Sp2.	5	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ke Np1	6	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ke Np2	16	@	-	-	-	-	@@@	@@	@@	@@	-	-
Ke Np3	7	@	-	-	-	-	@@@	@@	@@	@@	-	-
Ke Np4	43	-	-	-	-	-	@@@	@@	@@	@@	-	-

In Table 7 :-

(1) Though 7 strains of the first two groups down to Da Np agglutinate well at 1-50, of these only Gl Sp. gives one plus at 1 in 400. None of these two groups absorb.

(2) From the homologous coccus Coh Sp down to Br Np. both agglutination and absorption are correspondingly well marked, in all nine the reactions being practically perfect.

Hu Sp. is slightly exceptional but reference to Table 5 will show that both reactions are greater with Coh Sp Serum than with Wl Sp serum.

(3) Ca Sp. and Br Np. are found to agglutinate to full titre and to absorb the agglutinins perfectly whereas in Table 5 it is seen that they only agglutinate with Wl Sp serum to 1 in 50 and 1 in 100 but absorb as well as any member of Type A except the homologous coccus. They must therefore be classed as Types C and A.

(4) None of the Ke strains either agglutinate or absorb.

Table 8 which follows is of special interest:-

(1) As the serum Ke Spl was prepared from a pathogenic meningococcus which does NOT agglutinate with any of the Type Sera 1 to IV supplied by Lt.-Col. Mervyn Gordon, nor with sera Types A, B. and C above.

Two Spinal and four nasopharyngeal strains which agglutinated to 1 in 400 or 1 in 800 all completely absorbed the agglutinins from the serum prepared from the first spinal strain.

(2) Agglutination with the 30 other cocci was practically nil as was also absorption.

Table 9 is a summary of the preceding four tables giving the titre at which complete agglutination or absorption takes place.

TABLE 8.

Serum Ke Spl Type D (?? Gordon. Not Type IV)

Test Coccus	No.	AGGLUTINATION					ABSORPTION. v Homologous Coccus.				v Test Coccus.	
		1-50	100	200	400	800	100	200	300	400	100	200
Wl Sp.	2	-	-	-	-	-	@@@	@@	@@	@@	-	-
Mul.Sp.	4	-	-	-	-	-	@@@	@@	@@	@	-	-
Bl Sp.vii	9	-	-	-	-	-	@@@	@@	@@	@	-	-
Gl Sp.	10	-	-	-	-	-	@@@	@@	@@	@	-	-
Tl Sp.	32	-	-	-	-	-	@@@	@@	@@	@@	-	-
W2 Sp.	3	-	-	-	-	-	@@@	@@	@@	@@	-	-
W2 Np.	11	-	-	-	-	-	@@@	@@	@@	@	-	-
Clen.Np.	24	-	-	-	-	-	@@	@@	@	-	-	-
Dyb.Np.	23	-	-	-	-	-	@@	@@	@@	@	-	-
Mor.Np.	22	-	-	-	-	-	@@@	@@	@@	@	-	-
Key Sp.	13	-	-	-	-	-	@@@	@@	@@	@@	-	-
Sh Np.	45	@	@	-	-	-	@@	@@	@@	@	-	-
Ho Sp.	12	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ho Np.	34	-	-	-	-	-	@@@	@@	@@	@	-	-
Mc Np.	31	-	-	-	-	-	@@@	@@	@@	@	-	-
Ma Np.	29	-	-	-	-	-	@@@	@@	@@	@	-	-
Du Np.	27	-	-	-	-	-	@@	@@	@@	@	-	-
Ph Np.	30	-	-	-	-	-	@@@	@@	@@	@	-	-
Br Np.	35	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ch Np.	25	-	-	-	-	-	@@@	@@	@@	@@	-	-
Da Np.	41	-	-	-	-	-	@@@	@@	@@	@@	-	-
Coh Sp.	18	-	-	-	-	-	@@@	@@	@@	@@	-	-
La Np	46	@	@	-	-	-	@@@	@@	@@	@@	-	-
Ga Sp.	15	-	-	-	-	-	@@@	@@	@@	@@	-	-
N84 Sp.	20	@	-	-	-	-	@@@	@@	@@	@@	-	-
Gil Sp.	8	-	-	-	-	-	@@@	@@	@@	@@	-	-
Ne Np.	39	@	@	-	-	-	@@@	@@	@@	@@	-	-
Hu Sp.	19	-	-	-	-	-	@@	@@	@@	@	-	-
Ca Sp.	28	-	-	-	-	-	@@@	@@	@@	@@	-	-
Br Np.	26	@	-	-	-	-	@@@	@@	@	@	-	-
Ke Spl	1	@@@	@@	@@	@@	@	-	-	-	-	-	-
Ke Sp2	5	@@@	@@	@@	@@	@	-	-	-	-	-	-
Ke Npl	6	@@	@@	@@	@	-	-	-	-	-	-	-
Ke Np2	16	@@@	@@	@@	@	-	-	-	-	-	-	-
Ke Np3	7	@@@	@@	@@	@	-	-	-	-	-	-	-
Ke Np4	43	@@@	@@	@@	@	-	-	-	-	-	-	-

TABLE 9.

COMPLETE AGGLUTINATION TITRE OF TEST COCCUS AND OF HOMOLOGOUS COCCUS ON ABSORPTION WITH CORRESPONDING COCCUS.

Test Coccus	Serum W1 Sp. Test Coccus Aggl.	Homol. Coccus on abs.	Serum W2 Sp. Test Coccus Aggl.	Homol. Coccus on Abs.	Serum Coh. Sp. Test Coccus Aggl.	Homol. Coccus on Abs.	Serum Ke Sp. Test Coccus Aggl.	Homol. Coccus onAbs.
W1 Sp.	400	-	-	400	-	400	-	400
Mul Sp.	200	@	-	400	50	400	-	300
B1 Sp.vii	400	@	@	400	100	400	-	300
G1 Sp.	400	@	@	400	200	400	-	300
T1 Sp.	200	100	-	400	50	400	-	400
W2 Sp.	-	400	400	-	@	400	-	400
W2 Np.	-	400	400	-	-	400	-	300
Clen. Np.	-	400	400	-	@	400	-	200
Dyb. Np.	@	400	400	-	@	400	-	300
Mor.Np.	@	400	400	-	100	400	-	300
Key Sp.	-	400	200	-	-	400	-	400
Sh Sp.	-	400	400	@	-	400	@	300
Ho Sp.	-	400	200	-	-	400	-	400
Ho Np.	-	200	200	-	-	400	-	300
Mc Np.	-	400	200	-	-	300	-	300
Ma Np.	-	300	200	-	50	300	-	300
Du Np.	-	300	200	-	-	400	-	300
Ph Np.	-	300	200	-	-	400	-	300
Br Np.	@	300	200	-	50	400	-	400
Ch Np.	-	300	100	@	@	400	-	400
Da Np.	@	300	200	100	@	400	-	400
Coh Sp.	50	400	@	400	400	-	-	400
La Np.	50	200	100	400	400	-	@	400
Gr Sp.	100	200	@	300	400	@	-	400
N84 Sp.	100	300	@	400	400	@	@	400
Gil Sp.	200	200	-	400	400	@	-	400
Ne Np.	200	200	@	300	400	-	@	400
Hu Sp.	100	300	@	300	200	50	-	300
Ca Sp.	50	@	50	200	400	-	-	400
Br Np.	100	@	50	300	400	-	@	200
Ke Sp1	-	400	@	400	-	400	400	±
Ke Sp2	@	300	@	400	-	400	400	-
Ke Np1	-	300	-	300	-	400	200	-
Ke Np2	-	300	-	400	@	400	200	-
Ke Np3	@	400	@	400	@	400	200	-
Ke Np4	-	300	-	400	@	400	200	-

T A B L E 10.

Serum W1 Sp. Type A (Type III Gordon).

Test Coccus.	No.	AGGLUTINATION					ABSORPTION. v Homologous Coccus.				v Test Coccus.	
		1-50	100	200	400	800	100	200	300	400	100	200
Bro Np.1	21	@@	@	-	-	-	@@@	@@	@@	@@	-	-
Bro Np.3.	38	@@@	@@	@	@	-	@@	@@	@	-	-	-
Bro Np.4.	14	@@	@@	@@	@	-	@@@	@@	@@	@@	-	-
Bro Np.5.	44	@@	@@	@	-	-	@@	@@	@	-	-	-
Dev. Np.1.	47	@@	@	-	-	-	@@	@@	@	-	-	-
Dev. Np 4.	17	@@	@@	@	-	-	@@	@@	@@	@	-	-
Law Np.	42	@@	@	-	-	-	@@	@@	@@	@	-	-
Sta Np.	40	-	-	-	-	-	@@	@@	@@	@	-	-
Mon Np.	36	@@	@	@	-	-	@@	@@	@	-	-	-
Ham Np. (Flavus iii)	37	@@	@@	@@	@	-	@@	@@	@@	@	-	-
W1 Sp.	2	@@@	@@	@@	@@	@	-	-	-	-	-	-

T A B L E 11.

Serum W2 Sp. Type B (Type III Gordon).

Test Coccus.	No.	AGGLUTINATION					ABSORPTION. v. Homologous Coccus.				v Test Coccus.	
		1-50	100	200	400	800	100	200	300	400	100	200
Bro Np.1	21	@@	@	-	-	-	@@@	@@	@@	@@	-	-
Bro Np.3.	38	@@@	@@	@@	@	-	@@	@@	@@	@	-	-
Bro Np.4.	14	@@	@@	@	-	-	@@@	@@	@@	@@	-	-
Bro Np5.	44	@@@	@@	@@	@@	@	@@	@@	@@	@	-	-
Dev.Np1.	47	@@	@@	@@	@	-	@@	@@	@@	@	-	-
Dev.Np4.	17	@	-	-	-	-	@@@	@@	@@	@@	-	-
Law Np.	42	@	-	-	-	-	@@@	@@	@@	@@	-	-
Sta Np.	40	@@	@	-	-	-	@@@	@@	@@	@@	-	-
Mon Np.	36	@@	@	@	-	-	@@@	@@	@@	@@	-	-
Ham Np. (Flavus iii)	37	@@	@@	@@	@	-	@@@	@@	@@	@@	-	-
W2 Sp.	3	@@@	@@	@@	@@	@	-	-	-	-	-	-

TABLE 12.

Serum Coh Sp. Type C. (Type 1 Gordon).

Test Coccus	No.	AGGLUTINATION.					ABSORPTION. v. Homologous Coccus.				v. Test Coccus.	
		1-50	100	200	400	800	100	200	300	400	100	200
Bro Npl	21	-	-	-	-	-	@@@	@@	@@	@@	-	-
Bro Np3	38	-	-	-	-	-	@@@	@@	@@	@@	-	-
Bro Np4	14	@	-	-	-	-	@@@	@@	@@	@@	-	-
Bro Np5	44	-	-	-	-	-	@@@	@@	@@	@@	-	-
Dev Npl	47	@@	@	-	-	-	@@@	@@	@@	@@	-	-
Dev Np4	17	@	@	-	-	-	@@@	@@	@@	@@	-	-
Law Np	42	@@	@	-	-	-	@@@	@@	@@	@@	-	-
Sta.Np.	40	@	@	-	-	-	@@@	@@	@@	@@	-	-
Mon Np.	36	@	@	-	-	-	@@@	@@	@@	@@	-	-
Ham Np (Flavus iii)	37	@@	@@	@	-	-	@@@	@@	@@	@@	-	-
Coh. Sp.	18	@@@	@@	@@	@@	@	-	-	-	-	-	-

TABLE 13.

Serum Ke Spl Type D (?? Gordon NOT Type iv)

Test Coccus	No.	AGGLUTINATION					ABSORPTION. v. Homologous Coccus.				v Test. Coccus.	
		1-50	100	200	400	800	100	200	300	400	100	200
Bro Npl	21	-	-	-	-	-	@@	@@	@	-	-	-
Bro Np3	38	-	-	-	-	-	@@@	@@	@@	@@	-	-
Bro Np4	14	@@	@	-	-	-	@@	@@	@@	@	-	-
Bro Np5	44	@@	@	@	-	-	@@	@@	@	-	-	-
Dev Npl	47	-	-	-	-	-	@@@	@@	@@	@	-	-
Dev Np4	17	-	-	-	-	-	@@	@@	@@	@	-	-
Law Np	42	@@	@	-	-	-	@@@	@@	@@	@@	-	-
Sta Np.	40	@	@	-	-	-	@@@	@@	@@	@	-	-
Mon Np.	36	@	@	-	-	-	@@	@@	@@	@	-	-
Ham Np. (Flavus iii)	37	@@	@@	@	-	-	@@@	@@	@@	@@	-	-
Ke Spl	1	@@@	@@	@@	@@	@	-	-	-	-	-	-

TABLE 14.

COMPLETE AGGLUTINATION TITRE OF TEST COCCUS AND OF HOMOLOGOUS COCCUS ON ABSORPTION WITH CORRESPONDING COCCUS.

Test Coccus.	Serum W1 Sp. Test Coccus Aggl.	W1 Sp. Homol. Coccus on Abs.	Serum W2 Sp. Test Coccus Aggl.	W2 Sp. Homol. Coccus on Abs.	Serum Coh Sp. Test Coccus Aggl.	Coh Sp. Homol. Coccus on Abs.	Serum Ke Sp Test Coccus Aggl.	Ke Sp Homol Coccus. on Abs.
Bro Np1.	50	400	50	400	-	400	-	200
Bro Np3.	100	200	200	300	-	400	-	400
Bro Np4.	200	400	100	400	@	400	50	300
Bro Np5.	100	200	400	300	-	400	50	200
Dev.Np1.	50	200	200	300	50	400	-	300
Dev.Np4.	100	300	@	400	@	400	-	300
Law Np.	50	300	@	400	50	400	50	400
Sta Np.	-	300	50	400	@	400	@	300
Mon Np.	50	100	50	400	@	400	@	300
Ham Np. (Flavus III)	200	300	200	400	100	400	100	400
Controls W1 Sp.	400	-	-	400	-	400	-	400
W2 Sp.	-	400	400	-	@	400	-	400
Coh.Sp.	50	400	@	400	400	-	-	400
Ke Sp.	-	400	@	400	-	400	400	-

Tables 10 to 13 constitute the "scrap-heap" of those cocci which cannot from the absorption test be placed in any of the types represented in the preceding tables. The first four came from the same ? contact and are chiefly of interest on account of the variability of reaction in cocci from the same man. The succeeding two also came from one other doubtful contact. The remainder were from civilians who could not be considered as close contacts of a case of C.S. fever. (A specimen of Flavus 111 has been included for comparison and will not be referred to further.) Nos. 21,42,40 and 36 are probably not meningococci at all and the others are probably a sub-group of Type B. For administrative purposes they were regarded as Meningococci. Patient "Bro" continued a carrier for 127 days.

The organisms in these tables have been separated in order to bring out as clearly as possible the grouping, but the tables containing them have been included to complete the series tested which was absolutely unselected and made up of cocci from various sources. A Summary of these 4 tables appears as Table 14.

6.DISCUSSION.

Since this work was started there have been marked differences of opinion between bacteriologists as to the value of the agglutination method. To Lt.-Col. Mervyn Gordon R.A.M.C. belongs the credit of having put this method on a practical basis. On

On the other hand a great deal of work was done by the Medical Officers of the Local Govt. Board and published (24) in a report in 1916 followed by further reports in 1917 (25). In the former of these two L.G.B. reports the general conclusion was that reliance should be based only on microscopical and cultural characters including fermentation tests. In the latter it was clearly shown that meningococci could be divided into two main groups serologically, which groups could again be subdivided serologically. A great mass of evidence was further accumulated to show that the serological reactions of Nasopharyngeal cocci with characters of Meningococci were so variable that identification by means of type sera would be erroneous.

It is therefore of interest to see how far the results given above bear out these contentions. Reference to preceding tables will show:-

(1) Though the use of agglutination with two type sera viz. Types B & C would have identified very nearly all the true Meningococci, at least three spinal strains from acute cases of C.S. fever would have been missed.

(2) Use of three types of sera viz. A, B & C identified both by agglutination and absorption all of the spinal strains with the single exception of Ke Sp. which was from a sporadic civilian case (as an exception conversely compare G1, under "Fermentation reactions" which though a definite pathogenic meningococcus of Type A, gave completely negative fermentation reactions).

(3) Excluding at present the Ke Strains, these three sera failed to identify doubtful nasopharyngeal strains from (a) two doubtful contacts and (b) three civilians who cannot be regarded as close contacts of a case of C.S. fever, whereas they identified by both agglutination and absorption tests, 17 nasopharyngeal cocci as meningococci from cases or close contacts.

(4) The agglutination test alone with three type sera will identify in a manner and degree satisfactory for most practical purposes true meningococci both spinal and nasopharyngeal, though confirmation is required from the absorption test in some few doubtful cases.

(5) Exceptions occur among meningococci causing sporadic cases and some nasopharyngeal cocci as is well illustrated by the Ke Sp and Ke Np series which has been thoroughly worked out with a view to emphasing this. These exceptions are specially interesting from a purely scientific point of view. They indicate possibly that under certain circumstances nasopharyngeal cocci of doubtful nature may take on virulence and become pathogenic, though the identification of these doubtful nasopharyngeal cocci has little to do with the detection of carriers of meningococci of known pathogenicity in an Epidemic. The development of sporadic cases is a rare occurrence compared with the rapid spread of cases of C.S. Fever during an epidemic due to carriers. And thus laboratory methods which detect nasopharyngeal meningococci capable of spreading the disease in an epidemic are of the utmost value even though these methods may not detect all the doubtful organisms referred to above.

(6) In this connection I desire briefly to mention the very similar conditions seen regarding other organisms such as Pneumococci, and the various types of B. Dysenteriae. Dochez and Avery (26) classify Pneumococci into four groups of which Group IV is a "scrap-heap" the members of which are on the borderland between a saprophytic and a pathogenic life. Lister (27) in this country has divided Pneumococci into Types A,B,C etc. to K, but recognises that B corresponds with Group II Rockefeller, C with Group I Rockefeller, E with Group III, and that the remainder (except Type A) are as a rule more more confined to sporadic cases though they may give rise to local epidemics. As an example of this I may state that Type F (included in Group iv) was one of the very virulent organisms during the Influenza Epidemic of 1918 in Cape Town.

Regarding B. Dysenteriae, the work of E.G. Murray (28) shows the same general principle viz. that serological methods can place by far the majority of true pathogenic B. Dysenteriae into one or other of the well marked groups, but that there are no hard and

and fast lines between these groups. The importance of this grouping is not only for identification for diagnostic purposes but for the manufacture of anti-sera for treatment purposes. With reference to the latter I need only refer to the work published by Gordon under the auspices of the Medical Research Committee (31).

There is no doubt in my mind that for practical purposes as a basis for study Gordon was wise in defining as Meningococci gram-negative diplococci recovered from the C.S. fluid of actual cases of C.S. fever. I adopted the same basis independently, and I feel that in a remarkable degree the work detailed above brings one to the same conclusions as those arrived at by Gordon & Murray. Reference should also be made to Tulloch (29). I venture to repeat this because owing to the interest attached to exceptions there is danger that the valuable main results be overlooked.

Regarding the exceptions, I consider the question is bound up with far greater ones viz. "When is an organism to be considered pathogenic?" and "What are the causes that lead to organisms, which have been multiplying more or less harmlessly in certain parts of the body for indefinite periods, at some time acquiring virulent pathogenic properties for that person or another?"

Though it is useful to detect the spread of of organisms actually in a virulent state during an

an epidemic, it is far more necessary to determine if possible how to prevent the transition from comparative harmlessness into active pathogenicity.

Bacteriologists using methods of identification of the Meningococcus similar to those above have found that high carrier rates are associated with overcrowding and other non-hygienic conditions and at the same time with a spread of C.S. Fever. (E.G. Glover R.A.M.C. Journal Vol. XXX No. 1 Jan. 1918 (32)). This suggests that, in addition to the more rapid transference of pathogenic strains, the transition indicated above is probably largely dependant on such non-hygienic factors. It is a process somewhat similar to the increase of virulence of organisms by passage, but on a finer scale and more difficult of detection. There is therefore need for good administration and especially for education of the public, not only to avoid infection but to limit the opportunities of the organisms for increasing their virulence. So many infections are via the upper respiratory tract that much may be done on these lines.

I have ventured to touch upon these matters because while I believe that the work recorded above illustrates the practicability and great value of such bacteriological investigations of cases and carriers, I feel that bacteriological, clinical and administrative efforts should all be parts of one harmonious whole.

"MISCELLANEOUS FACTS OF INTEREST".

1. Under "Preparation of Antimeningococcal sera" above, it was noted that one animal Hul Sp. during inoculations showed clinical appearances typical of Meningitis. The notes are as follows:-
"Conjunctivitis and general malaise noticed for some days; not taking food. Yesterday was worse and sat with head raised and drawn in. To-day, is definitely retracted and stiff neck present. When animal is raised by fur over back, the head and neck arch backwards instead of falling as is normal. There is complete loss of power of limbs though they move reflexly when stimulated. Sometimes attempt to raise animal results in clonic nodding movements of the head with neck still back and chin forward."

As this condition was clinically typical of Meningitis the animal was (1) bled from the Carotid (a) into lemco broth and other media and (b) some blood collected for serum (Titre found to be 1-1600). (2) The brain and spinal Cord were exposed aseptically: Cultures and smears were made from the subdural fluid from the lymph on brain and spinal Cord. The brain and cord were then removed and Cultures inoculated from emulsions of them.
(3) .25 c.c. of an emulsion of brain in .85% Saline were injected subdurally into another healthy rabbit trephined for the purpose.

There was intense congestion with flakes of lymph present but there was no well-marked pus to the naked eye.

RESULTS.

The smears showed presence of gram-negative diplococci with characters of Meningococci but in small numbers, with some pus cells.

The blood culture remained sterile as did also the other Cultures. The rabbit injected subdurally did not develop symptoms of the disease. Though the evidence is thus partial, attention is drawn to two facts:-

(1) The animal was undoubtedly suffering from meningitis clinically, produced by intravenous injections of living cultures of meningococci.

(2) Meningococci were found in several slides from lymph of both brain and spinal cord.

These facts were corroborated by Col. G.W. Robertson, Government Bacteriologist and J.W. Campbell, Laboratory Assistant. It is therefore considered that meningitis was produced by intravenous injections of living meningococci in this animal.

It appears possible that animals do not show symptoms of meningitis when inoculated intravenously because they die in the Septicaemic stage. The immunisation of this animal may have caused the disease to become less acute and allowed the development of meningeal signs to take place.

11. In the case of B1, meningococci were obtained from seven specimens of C.S. fluid. Agglutination reactions with these showed practically no variation from one another. Note also the close similarity of

of reactions of the Ke series. Agglutination was performed with 18 Nasopharyngeal strains from patient Ke with four sera, the results being almost the same in each case. It is noteworthy that a nasopharyngeal strain from a case of C.S. Fever usually agglutinates fully to a slightly lower titre than the corresponding spinal Strain.

111. Regarding media, so much has been written elsewhere that it is unnecessary to do more than briefly state those found most satisfactory.

(1) For primary cultures from C.S. fluid the most important factor when the number of cocci is small, is incubation of the fluid itself at 37°C for 12 to 24 hours. The collection must therefore be done in a perfectly aseptic manner. The natural or centrifugalised deposit is then plated on agar or nasgar with two or three drops of blood on the surface. It is well to wait till the blood has clotted and to remove the fibrin before spreading.

(2) Primary cultures from nasopharyngeal swabs are made directly on surface-blood-agar plates.

(3) Defibrinated rabbit-blood nasgar or agar with addition of 1% Glucose was found the best for coaxing feebly growing strains and for procuring a large growth.

(4) Vedder's corn-flour starch agar was used as slopes or plates for producing cocci for emulsions and as stabs for keeping strains alive. The latter should be covered with a rubber cap to prevent evaporation. The plugs should be flamed before replacement in the tube to prevent possible development of mould spores. Meningococci will keep alive in this manner for 6 weeks with safety and up to three months in many cases, if incubated at 37°C.

Owing to the fact that I personally captured Peptone and Nutrose in German territory I have only recently adopted Trypagar, which however is excellent.

(5) The same cornflour starch agar with addition of 1% Sugars and of litmus is the most useful for fermentation tests.

IV. During the past two years the above methods have been used as a routine in the Govt. Bacteriological Laboratory, Cape Town and 15 Meningococci from sporadic cases have been tested with Type sera. The results are entirely confirmatory, it having been possible to place them according to their agglutination reactions as one or other of the types. In one case affinnity was shown between Types II and III.

I have not included this work in this report as I wish it to be purely a report of work done from June 1916 to October 1917. I sincerely regret that extreme pressure of Military work has prevented the completion of the report till now though all the data had been carefully collected by then.

V. I desire to draw attention to the fact that this test is not one purely of agglutination of cocci; in reality it is a combination of agglutination with a Precipitin test. This explains why a temperature of 55°C is especially favourable. No doubt this temperature favours the combination of cocci with agglutinins and that the convection currents set up are useful, but the large bulk of the deposit cannot be accounted for by the massing of all the cocci when only 1000 million are used. From work done in the University of Edinburgh it was known to me that 55°C was the temperature most suitable for Precipitin tests, and that the Precipitum came almost entirely from the Serum, only very dilute extracts of organisms being required. These "extracts" are

are present in the above test and the presence of the cocci themselves also, does not prevent the test being chiefly one of precipitation as well as of agglutination. This also accounts for the larger bulk of deposit in those tubes containing the lower dilutions of serum, and for the larger bulk of deposit in tubes of immune serum compared with that of normal serum in a "Flavus reaction".

VI. It is unnecessary to quote in detail the results of the tests made by Col. Gordon upon the 37 killed coccal emulsions sent by me. In the R.A.M.C. Journal Jan. 1918 at the conclusion of his article(30) he reports "Captain E Douglas Pullon, (Assistant to the) Government Bacteriologist at Cape Town investigated a series of Meningococci obtained from cases of Cerebrospinal fever in S.Africa, and having differentiated them with agglutinating sera prepared by himself, forwarded suspensions of these cocci to the Central Laboratory for examination. The suspensions arrived a few weeks ago and were tested against the four univalent sera in the routine manner, with the result that of sixteen meningococci from cerebrospinal fluid nine were specimens of Type 1, five were specimens of Type 2 and two were specimens of Type 3. No example of Type 4 was found. On reference to the enclosure in Captain Pullon's letter giving his own results, it was seen that the classification of these cerebrospinal fluid strains effected by our sera was identical with that made by him; the only difference being that his

his Group A was our Type 3, his Group B, Type 2, and his Group C, Type 1. There is good reason to believe, therefore, that a limit obtains with regard to the diversity of meningococci; and that at any rate the most important pathogenic members of the group have now been defined."

VII. Col. Gordon supplied me with his Type Sera I to IV and homologous cocci. I confirmed the above in para VI by agglutinating my own cocci with his sera. Further, his Type I coccus agglutinates to 1 in 400 with my Serum Type C., his Type II coccus with my Serum Type B and his Type III coccus with my Serum Type A. His Type IV serum did NOT agglutinate Kel Sp or any other of my cocci. (I had thought previously that Ke might be Type IV)

Though it is natural that I regret that Military work has prevented the completion of this paper before the publication of similar results, it is very gratifying to be able to bring forward results which so strongly confirm how much can be done with the agglutination and absorption tests in the identification of the Meningococcus. And I would add that the work itself was completed by the end of 1917.

In conclusion I have the greatest pleasure in thanking Lt.-Col. G.W. Robertson S.A.M.C., Government Bacteriologist, for providing the necessary facilities and for his kindly criticism ; to Dr.

Dr. A Jasper Anderson, Medical Officer of Health for City of Cape Town, who treated cases and carriers and supplied much of the material: and also to Mr. J.W. Campbell, Laboratory Assistant, for his technical assistance which he gave willingly, often at personal inconvenience, in a true scientific spirit.

S U M M A R Y.

(1) The percentage of Carriers amongst 921 close contacts of C.S. fever was 8.79%; and amongst 451 non-contacts 1.99%.

(2) A condition clinically resembling meningitis was produced in a rabbit by intravenous injections of living meningococci.

(3) Of 91 Meningococci, 87 (32 C.S.F. & 55 Nasoph.) fermented Glucose and Maltose only; 4 (1 C.S.F. & 3 Nasoph.) fermented Maltose only. 3 Flavus III fermented Glucose and Maltose only. 1 C.S. fluid meningococcus fermented NO "Sugars".

(4) By agglutination tests 21 out of 24 meningococci were classified into Types A,B, C and D. Types A and C are related.

(5) Agglutination and absorption tests with three Type sera A,B and C identified, (a) 15 out of 16 Spinal strains, the exception Ke Sp being from a sporadic case. (b) 17 out of 25 nasopharyngeal cocci, the exceptions being of doubtful nature.

(6) Types C,B, and A correspond with Gordon's Types I, II and III and were arrived at independently.

(7) Agglutination tests (with rarely necessary absorption tests) with Type Sera, though not infallible, provide a method of the greatest practical value for identification of the Meningococcus for diagnosis and specific serum treatment.

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