

A BACTERIOLOGICAL INVESTIGATION OF ORGANISMS RESEMBLING THE MENINGOCOCCUS FOUND BY EXAMINATION OF THE NASOPHARYNX OF PERSONS WHO HAD NOT BEEN IN CONTACT WITH PATIENTS SUFFERING FROM CEREBRO-SPINAL FEVER<sup>1</sup>.

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## INTRODUCTION.

THE presence of the meningococcus, or an organism indistinguishable from it by any known tests, in the naso-pharynx of persons who have not suffered from cerebro-spinal fever, nor been in contact with other persons suffering from this disease, has been studied during the year 1915 by Drs Eastwood, Griffith and Scott<sup>1</sup>. Their results showed that in the London District an unexpectedly large proportion of persons examined were carriers of this organism. In view of these results the Medical Officer of the Local Government Board considered it desirable that a further investigation should be made in a different district, where the incidence of cerebro-spinal fever had been low, and where, consequently, chances of spread of the meningococcus by direct contact with the disease would be small. The Eastern Counties seemed to meet these requirements, and accordingly, after the War Office had loaned my services to the Local Government Board for this purpose, I was instructed by the Board's Medical Officer to make investigation on the subject in question amongst the inhabitants of Cambridge, Norwich, and the surrounding areas.

I am greatly indebted to Drs Eastwood, Griffith and Scott for their valuable help during the investigation; by utilising their experiences fully in regard to media and methods a good deal of preliminary work was avoided.

The earlier part of my work, from June to October, was done in the Pathological Laboratories of the Medical Schools at Cambridge, for the use of which I wish to thank Professor Woodhead.

I am also much indebted for help, at Cambridge, to Dr Laird, the Medical Officer of Health of the Borough, Captain J. C. Graham, Dr Varrier Jones and Dr Carter (of Soham). At Norwich, Dr Cooper Pattin (the Medical Officer of Health), Dr Claridge, and Dr Long gave me every assistance. Dr Claridge, Pathologist to the Norfolk and Norwich Hospital, very kindly placed his laboratory at my disposal during my visit to the city.

The latter part of the investigation, from October, 1916, to February, 1917, was devoted to the serological examination of the strains found and was done in the Board's Laboratories.

<sup>1</sup> *Journ. of Hygiene*, xv. pp. 405-484.

INCIDENCE OF CEREBRO-SPINAL MENINGITIS AMONGST THE  
POPULATION IN THE AREAS INVESTIGATED.

The following summary, which is prepared from information kindly supplied by the Medical Officers of Health of Cambridge and Norwich, gives details of all cases notified during 1916.

*Cases of Cerebro-spinal Fever notified in Cambridge and Norwich  
between Jan. 1 and Dec. 31, 1916.*

	Patient	Date	Address	Termination	Notes
Cambridge (Pop. 40,027)	Girl (age 6)	Mar. 27th	Outskirts of town	Recovered	Only father and mother came in contact. No examination of throats was made
	Girl (age 13)	Mar. 20th	Outskirts of town	Died	First notified as enteric fever and removed to general hospital. No examination made
Norwich (Pop. 121,478)	Woman	Feb. 3rd	Town	Recovered	The naso-pharynx of ten contacts examined and one found positive
	Woman	Feb. 15th	Town	Died	Two contacts examined, none found positive
	Soldier	Feb. 28th	Town	Recovered	Eight contacts examined, five found positive
	Man	Mar. 11th	Town	Recovered	Ten contacts examined, none found positive
	Girl	Apl. 18th	Town	Died	Four contacts examined, none found positive
	Man	June 8th	Town	Recovered	Eight contacts examined, none found positive
	Soldier	Nov. 7th	Town	Died	Contacts swabbed, nega- tive

No cases were notified at either place whilst my work there was in progress, and only one subsequently.

Considering the large population of these places and the small number of cases of the disease which occurred before the investigation had begun, it will be agreed that the number of persons who can have come in *direct* contact with patients must have been very small. How far *indirect* contact may have occurred it is impossible to say.

Attempts were made to include amongst the persons chosen for swabbing individuals of different classes, living under different circumstances and in different states of health.

The swabbings were carried out in three batches:

(1) Two hundred swabs were taken in Cambridge during June and July, 1916, mostly from the naso-pharynx of out-patients at Addenbrooke's Hospital and their friends. Nearly all these patients were attending the eye department for slight defects and may, therefore, for the purposes of the investigation, be classed as normal people, since they were able to go about and in most cases do their work.

A few, however, taken at a Tuberculosis Clinic, came from patients suffering from phthisis in different stages, and the health of these patients must be classed as "impaired."

(2) A hundred swabs were taken at Norwich during August, 1916, from the naso-pharynx of medical and surgical out-patients and their friends attending the Norfolk and Norwich Hospital. Many of these patients had impaired health but all were well enough to be up and about.

(3) A hundred swabs were taken during October, 1916, amongst the employees at a factory in Cambridge where scientific instruments, etc., are manufactured. These employees were all in good health and, as they were all earning good wages, it may be assumed that their home circumstances were on the whole much more comfortable than those of the other two groups of persons swabbed.

#### METHODS ADOPTED IN THE INVESTIGATION.

##### *Method of Swabbing.*

Success in recovering such a delicate organism as the meningococcus depends, I think, a good deal on the amount of care given to the taking of the swab. A simple form of swab is efficient if properly prepared. In this investigation I used a plain aluminium or tinned-wire rod; the end around which the wool was wrapped was bent round to an angle. Experience soon showed that this angle must be carefully adjusted; if it was too obtuse, the handle-end of the swab impinged on the lower teeth and the wool-wrapped end could not pass properly behind the soft palate, since its point struck the posterior wall of the pharynx. The angle should be slightly larger than a right angle. Wool was wrapped round the bent end so that no metal was exposed, and the part protruding beyond the end was cut off with scissors; thus the swab ended in a flat padded surface to which mucus easily adhered. I preferred such a swab to the guarded kind, as one could guide the point with more accuracy; there was also a larger surface of wool brought in contact with mucous membrane.

The patient was placed in a good light and instructed to open the mouth as wide as possible. The tongue was depressed by a wooden spatula placed as far back as convenient. The swab then being held ready on the dry upper surface of the spatula, the patient was told to phonate the sound "ah," and, directly the soft palate rose as a result of this action, the bent end of the swab was passed up behind the soft palate. In carrying out this process the outer part of the wool is rubbed on the back wall and vault of the naso-pharynx, and, if carefully conducted, there is no possibility of contamination by saliva. That surface of the swab which had come in contact with the naso-pharynx was then rubbed over an ascitic-agar plate, taking care to transfer to the surface of the medium any beads or threads of mucus which had adhered to the wool.

#### *Media and Cultivation of Swabs.*

The plate to which the mucus was transferred contained ascitic-agar made from ordinary nutrient agar (reaction + 8) containing 2.5 per cent. agar. This, after being melted and cooled to 55° C., had received the addition of sterile ascitic fluid in the proportion of one part ascitic fluid to three parts nutrient agar.

The plates were taken to the laboratory without delay; but it was not found essential to keep them at blood temperature in a portable incubator, as some investigators have stated. The mucus was spread out on the plate by means of a bent glass rod, care being taken to tease it out as thoroughly as possible. The same glass rod was then wiped lightly over the surface of a second plate. The rod should only pass once over the surface, and a large plate is obviously better than a small one. This second plate contained Kutscher's medium prepared as follows: to a broth made from fresh human placenta, 500 grms. to the litre, are added nutrose 2 per cent., glucose 1 per cent., peptone (Chapoteaut) 1.5 per cent., and agar 2.5 per cent.; the reaction is brought to + 8 (Eyre's scale) after steaming. Tubes containing a convenient amount are melted, cooled to 55° C., and sterile filtered ox serum is added in the proportion of one part to three; the medium is then ready to pour into the Petri dish.

The plates were examined after 24 hours and after 48 hours; if the second plate was spread as described above, a sufficient number of colonies, not overcrowded, was obtained almost invariably. The primary ascitic-agar plate was usually overcrowded with colonies, and was only examined when the Kutscher plate contained very few colonies.

*Examination of Cultures and Preservation of Strains.*

After some experience the colonies of meningococcus-like organisms on Kutscher's medium can be distinguished readily, even by unaided sight, in a mixed culture such as is obtained from a throat swab. Their colour is the bluish-grey of tobacco-smoke. After 24 hours growth they are about 2 mm. in diameter, translucent, and with a lens may appear faintly granular. They are slightly raised, and the margins are regular. After 48 hours they are about 4 mm. in diameter, or even more where there is room for free growth; they preserve their original colour, translucency and faint granularity; they often show an annular appearance due to variations in thickness between the centre and circumference. In some strains the edges of the colonies may become slightly irregular and slight striation may be noticed. Colonies do not fuse readily one with another, and in the case of some strains a distinct facetting may be noticed where two or more colonies grow in contact. When emulsified, as a rule they dissolve easily like paint; some strains may be slightly glutinous and some tend to adhere slightly to the medium. This latter characteristic appears to depend on certain qualities of the media. Some strains in subculture may assume a faintly yellow tint. These slight variations were also noticed in strains of meningococcus of cerebrospinal origin.

When the meningococcus-like organisms were present in a throat culture, they often formed a large proportion of the colonies on the plate and sometimes they were present in almost pure culture. This appears to indicate that these organisms find a suitable *habitat* in the naso-pharyngeal mucus and are well able to hold their own in competition with the other flora.

Strains retained for further examination were sown on slopes of egg medium, on which they remain alive usually for several months. This medium is prepared as follows: new-laid eggs with shells unbroken are placed one at a time in boiling water for 15 seconds, thus coagulating and sterilising the most external layer of albumen. The shells are then carefully broken on the edge of a sterile measuring cylinder, and the contents allowed to fall in without contamination. The amount is noted, and the contents of the cylinder are poured into a large sterile flask, which is shaken until all the yolks are broken. Sterile normal salt solution, in the proportion of one part to three parts of egg, is then added. The mixture is syphoned into test tubes and sterilised by inspissating in moist heat at 85° C., for two hours on each of two successive

days. The plugs of the tubes are sealed with melted paraffin to prevent the medium drying.

#### *Identification of the Meningococcus.*

Satisfactory methods for identifying the meningococcus cannot yet be said to be established. There is no practical test of pathogenicity and one is compelled to fall back on cultural and serological tests, in regard to which a definite standard of identification has not been agreed upon. Where the organism is leading a pathogenic existence, knowledge of the locality whence the strain was isolated plays a strong part in influencing the decision. For example, meningococci and gonococci have many bacteriological characteristics in common, and even serologically can only be distinguished *in vitro* by tests which are not very definite; if, however, one is cultivated from cerebro-spinal fluid and the other from a urethral discharge, there is a valuable clue for differential diagnosis. But with an organism isolated from a throat, and perhaps living a saprophytic existence there, the locality from which it is isolated gives no clue to its differentiation, since many varieties of gram-negative cocci occur in this region.

#### *Cultural characters.*

These have been described above in connection with the growth on Kutscher's medium. On subculture, certain strains were found to become yellow and glutinous; such strains often gave sugar reactions different from the meningococcus and invariably failed to agglutinate with the sera tested.

#### *Microscopical characters.*

Throat strains of meningococcus-like organisms seldom showed any difference in microscopical appearance from meningococci of cerebro-spinal origin. The presence of tetrads and larger groups of cocci varies, and seems to depend largely on conditions of growth.

#### *Fermentation of sugars.*

Strains were tested as regards their action on glucose, maltose and saccharose. The medium used was prepared according to Lingelsheim's formula. To 210 c.c. of ordinary 2.5 per cent. nutrient agar which has been melted and cooled to 55° C. are added 70 c.c. ascitic fluid, 20 c.c. of 10 per cent. solution of the required sugar, and sufficient Kubel and

Tiemann's litmus to give a good blue colour, all these latter solutions being heated to a temperature of 55° C. The medium is then syphoned into test tubes and allowed to cool in a sloped position.

On such slopes the characteristic growth of the meningococcus is readily visible, and the production of slight acidity is easily detected. A recently isolated, vigorous strain of meningococcus will usually show definite acidity on glucose and maltose slopes in 24 hours, but no acidity can be obtained on saccharose slopes. Strains that have been stored and subcultured several times may fail to produce acidity with either glucose or maltose or perhaps with both.

The sugar reactions of the great majority of the meningococcus-like organisms isolated from the throat resembled the reactions of meningococci of cerebro-spinal origin. A few were found that fermented saccharose; these did not agglutinate with the sera tested. A certain number when freshly isolated failed to give acid with either glucose or maltose; these, for the most part, were difficult to emulsify, and suspensions rapidly showed spontaneous sedimentation. All but two failed to show satisfactory agglutination with the sera used. These organisms were probably related to the type which has been described under the name *M. catarrhalis*.

#### *Serological tests; preparation of immune sera.*

Young rabbits weighing 1,500 to 2,000 grms. were inoculated intravenously at intervals of 5-7 days with increasing doses of live 24 hour ascitic-agar slope cultures. A satisfactory series of doses was found to be a half slope for each of the first two inoculations, a whole slope for each of the next two inoculations, and a slope and a half for each of the next two. In about six weeks to two months a serum which gave a titre for the homologous organism of at least 1:800 was usually obtained.

Nine monovalent sera were prepared, seven from cerebro-spinal and two from naso-pharyngeal strains.

#### *Preparation of suspensions of cocci.*

The whole of the growth from a 24 hour old ascitic-agar slope was spread by means of a glass spreader over the surface of two or three Petri plates of ascitic-agar. After 24 hours' incubation the growth was scraped off and weighed and a suspension of the required strength

was made up by adding sufficient phenol-saline solution (.5 per cent. phenol in normal saline).

For ordinary agglutination tests, the suspension was always used in a strength of 2 mg. to the c.c., making thus 1 mg. to the c.c. in the mixture of suspension and serum.

Contrary to the experience of some workers, it was found that suspensions made from growth on a medium containing ascitic fluid were not markedly less agglutinable than suspensions made from a growth on simple nutrient agar.

#### *Conduction of agglutination tests.*

The macroscopic method was always employed. The serum was diluted to strengths of 1 : 50, 1 : 100, 1 : 200 and 1 : 400; thus, when an equal quantity of coccal suspension was added, the serum was present in double this dilution, viz. 1 : 100, 1 : 200, 1 : 400, and 1 : 800. Sera were not tested in higher dilution. Half a cubic centimetre of each dilution was placed in a set of small test tubes arranged in series, and to each was added half a cubic centimetre of the suspension of the coccus to be tested. Racks holding the tubes were placed in an incubator at 55° C. for 24 hours, after which they were taken out and allowed to stand for 24 hours before reading the results.

#### RESULTS OF THE INVESTIGATION.

##### *Details respecting persons found to be carrying organisms indistinguishable from the meningococcus.*

The following table (Table I) gives certain details respecting the persons yielding colonies in the primary plate which resembled the meningococcus. The numbers in the left-hand column were given in sequence to each person in the order examined. In the column headed "Sugar reactions"  $G > M$  indicates that stronger acidity was produced with glucose than with maltose, while  $M > G$  indicates the reverse.  $G = M$  means equally marked acidity with the two sugars.

In the column headed "Highest agglutination" the arabic numerals indicate the highest dilution giving complete agglutination, while M I, M XIII, NP 108, etc., refer to the different monovalent sera with which this result was produced. *Vide* Table V for the agglutinating properties of these towards meningococci of cerebro-spinal origin.

TABLE I.  
*Details respecting persons yielding colonies in the primary plate which resembled the meningococcus.*

No.	Date	Sex	Age	Physical condition	Locality	Urban or Rural	No. of colonies on original plate	Sugar reaction	Sub-cultures	Highest agglutination
7	13. vi. 16	F.	34	Phthisis	Cambridge	Rural	Many	G. > M.	Like meningococcus	800 (M. i, etc.)
12	15. vi. 16	M.	29	"	Cambridgeshire	R.	Almost pure	M. > G.	"	800 (M. i.)
13	"	M.	24	Mitral disease	Cambridge	U.	Almost pure	M. = G.	"	800 (M. i, etc.)
18	17. vi. 16	F.	43	Normal	Ely	R.	Many	G. > M.	"	800 (M. i, etc.)
20	"	F.	48	"	Cambridgeshire	R.	Many	G. = M.	"	800 (M. i, etc.)
29	"	F.	45	"	"	R.	Many	G. = M.	Colonies yellow	Nil.
32	19. vi. 16	M.	53	"	Cambridge	U.	Many	G. = M.	Like meningococcus	400 (M. xiii, NP 108)
60	5. vii. 16	M.	49	Ophthalmia	"	U.	Large proportion	G. = M.	Colonies yellow	Nil.
65	"	M.	74	Normal	"	U.	Large proportion	?	Like meningococcus	800 (M. i, etc.)
68	"	F.	74	"	Ely	U.	Few	Ferments saccharose	Yellow colonies	Nil
70	"	M.	13	"	Cambridgeshire	R.	Few	G. = M.	Like meningococcus	800 (M. i, etc.)
76	7. vii. 16	M.	12	"	Cambridge	U.	Many	?	Strain lost	"
77	"	F.	41	"	"	U.	Few	M. > G.	Like meningococcus	Nil
90	10. vii. 16	M.	6	"	Cambridgeshire	R.	Many	Ferments saccharose	Yellow colonies	"
96	"	M.	6	Tonsils, etc., etc.,	Cambridge	U.	"	"	Like meningococcus	"
97	"	M.	13	Normal	"	U.	"	G. = M.	Died	"
99	"	F.	7	Congenital syphilis	"	U.	"	?	Like meningococcus	800 (M. iv, etc.)
100	"	M.	4	Adenoids	"	U.	"	"	Coloniae yellow	Nil
101	12. vii. 16	M.	29	Phthisis (early)	"	U.	"	Ferments saccharose	Like meningococcus	800 (M. iv, etc.)
106	"	M.	20	(advanced)	Patients at		Few	G. = M.	Like meningococcus	800 (M. iv, etc.)
108	"	M.	22	(advanced)	Bourn	Open	Almost pure	G. > M.	"	800 (M. iv, etc.)
109	"	M.	13	(early)	culosis colony	air life	Few	M. > G.	"	800 (M. i, M. iv)
112	"	M.	17	(early)	Cambridgeshire	R.	Pure culture	M. > G.	"	200 (NP 108)
117	19. vii. 16	M.	70	Normal	Cambridge	U.	Many	M. > G.	"	200 (M. xv, NP 108)
121	"	F.	49	"	"	U.	Few	?	Died	800 (M. i, etc.)
122	"	M.	61	"	"	U.	Many	M. > G.	Like meningococcus	Nil
129	"	M.	8	Keratitis	"	U.	Many	M. > G.	"	"
131	"	M.	75	Normal	Cambridgeshire	R.	Almost pure	M. > G.	"	400 (M. xv, NP 108)
136	21. vii. 16	F.	21	"	Cambridge	U.	One colony	M. = G.	"	"
139	"	M.	18	"	St Ives	R.	Many	M. = G.	"	"
141	"	M.	49	"	Cambridgeshire	R.	Few	Ferments saccharose	Colonies yellow	"
	"			"	"		"	M. > G.	Like meningococcus	800 (M. i, etc.)

146	21. vii. 16	F.	78	Normal	Cambridgeshire	R.	Many	G = M.	?	Like meningococcus .	Nil
148	"	M.	14	"	"	R.	Few	G = M.	?	Contaminated and outgrown	?
149	24. vii. 16	F.	5	"	Cambridge	U.	Few		?	Colonies yellow. Strain lost	?
150	"	M.	10	"	"	U.	Many		?	Like meningococcus .	400 (M. i, etc.)
153	"	F.	26	"	"	U.	Large proportion		?	Like meningococcus .	800 (M. xiii)
155	"	F.	38	"	"	U.	Large proportion		?	Colonies yellow .	Nil
161	29. vii. 16	F.	15	"	Cambridgeshire	R.	Few		?	Died .	?
164	"	F.	8	"	"	R.	Many	G = M.		Colonies yellow .	Nil
172	31. vii. 16	M.	12	"	Royston	U.	Many	G = M.		Like meningococcus .	800 (M. i, etc.)
174	"	F.	16	"	Cambridge	U.	Many	G = M.		Contaminated and outgrown	?
178	10. viii. 16	F.	27	"	"	U.	Many	G > M.	?	Died .	?
179	"	M.	32	"	"	U.	Large proportion		?	Like meningococcus .	800 (M. xiii)
181	"	F.	25	"	Cambridgeshire	R.	Few		?	Like meningococcus .	?
182	"	F.	36	Phthisis (early)	Cambridge	U.	Large proportion	G > M.		Like meningococcus .	800 (M. vi, M. xiii)
183	"	M.	45	Phthisis	Cambridgeshire	R.	Large	G = M.		Died .	?
184	"	M.	22	Normal	"	R.	Many	G > M.		Like meningococcus .	800 (M. i, etc.)
186	"	F.	15	Phthisis (early)	Cambridge	U.	Few	M > G.		Yellow colonies .	50 (NP 108)
188	"	F.	8	"	"	U.	Few	G = M.		Like meningococcus .	800 (M. xvi, M. xiii NP 108)
191	"	F.	38	Normal	"	U.	Large proportion		?	Died .	?
196	15. viii. 16	M.	69	"	Cambridgeshire	R.	Many	G > M.		Like meningococcus .	800 (M. xiii)
198	"	M.	36	"	"	R.	Large proportion		?	Died .	?
200	"	M.	56	"	"	R.	Many		?	Died .	?
201	23. viii. 16	F.	10	Lupus	Norfolk	R.	One colony		?	Died .	?
202	"	F.	32	Asthma	Norwich	U.	Many	G = M.		Like meningococcus .	200 (NP 108)
204	"	F.	38	Anaemia	"	U.	Many	G = M.		"	800 (M. xiii)
207	"	F.	46	Normal	"	U.	Many	G = M.		"	50 (M. xvi, M. xiii)
209	"	F.	33	Syphilis	"	U.	Large proportion	G = M.		"	200 (M. iv, NP 108, NP 235)

TABLE I—continued.  
*Details respecting persons yielding colonies in the primary plate which resembled the meningococcus.*

No.	Date	Sex	Age	Physical condition	Locality	Urban or Rural	No. of colonies on original plate	Sugar reaction	Sub-cultures	Highest agglutination	
211	23. viii. 16	F.	25	Epilepsy	Norwich	U.	Almost pure	?	Died	?	
212	"	F.	51	Goitre	"	U.	Many	G. = M.	Like meningococcus.	800 (M. i, etc.)	
214	"	F.	11	Normal	"	U.	Almost pure	G. = M.	"	800 (M. i, etc.)	
216	"	F.	26	Rheum., arthritis	"	U.	Almost pure	G. = M.	Like meningococcus.	800 (M. i, etc.)	
217	"	M.	45	Epilepsy	"	U.	"	?	Died	?	
222	26. viii. 16	M.	32	Phthisis	"	U.	Many	G. > M.	Like meningococcus.	800 (NP 235)	
223	"	F.	50	Normal	"	U.	Large proportion	G. = M.	"	800 (M. i, etc.)	
224	"	M.	8	Chorea	"	U.	Few	M. > G.	Too adherent to medium to emulsify	?	
227	"	F.	30	Normal	"	U.	Almost pure	G. = M.	Adherent to medium	?	
229	"	F.	40	Normal	"	U.	Large	G. = M.	Like meningococcus.	800 (M. i, etc.)	
232	"	F.	26	Chlorosis	"	U.	Large	G. > M.	"	800 (M. xiii, NP 235)	
235	"	F.	45	Normal	"	U.	Almost pure	G. > M.	"	800 (M. i, etc.)	
236	"	M.	65	Carcinoma	"	U.	Many	G. = M.	"	400 (M. xiv)	
237	"	M.	30	Normal	"	U.	Almost pure	Ferments saccharose	Colonies yellow.	Nil	
238	"	M.	15	Septic wound	"	U.	Large proportion	?	Adherent	?	
239	"	M.	15	Normal	"	U.	Many	?	Colonies yellow.	?	
242	"	M.	18	Normal	"	U.	Almost pure	G. = M.	Like meningococcus.	800 (M. i)	
244	28. viii. 16	M.	28	Deafness	"	U.	Few	Ferments saccharose	Very adherent to media	Nil	
245	"	M.	19	"	Soldiers under canvas at various camps and attending the Norfolk and Norwich Hospital for ear trouble	All R.	Many	G. = M.	Like meningococcus.	800 (M. xiii, NP 108, NP 235)	
247	"	M.	23	Otitis		"	"	G. = M.	"	800 (M. i, M. xiii)	
248	"	M.	21	Deafness		"	"	"	G. = M.	"	Nil
249	"	M.	24	"		"	"	"	G. = M.	Very adherent to media	50 (NP 235)
250	"	M.	30	Otitis	"	"	Almost pure	G. = M.	Like meningococcus.	800 (M. i, etc.)	
255	"	M.	20	Deafness	"	"	One colony	?	Died	?	
256	"	M.	29	Oorrhoea	"	"	Few	G. = M.	Like meningococcus.	800 (M. i, M. xiii)	
257	"	M.	46	Normal	"	U.	Many	G. = M.	Very sticky in sub-culture	?	
259	"	F.	5	Otitis	"	U.	Few	G. = M.	Too adherent to make emulsion	?	

262	28. viii. 16	M.	14	Normal		Norwich	U.	Large proportion	G. = M.	Like meningococcus.	800 (NP 108)
264	"	M.	18	"		"	U.	Few	G. = M.	Yellow colonies.	Nil
265	30. viii. 16	M.	26	Epilepsy		"	U.	Large proportion	G. = M.	Like meningococcus.	200 (NP 108)
267	"	F.	51	Myxoedema		"	U.	Few	?	Adherent	?
268	"	F.	18	Dyspepsia		"	U.	Large proportion	?	"	?
271	"	F.	29	Rheum. arthritis		"	U.	Large proportion	?	"	?
272	"	F.	36	"		"	U.	Few	G. = M.	"	200 (M. xv, NP 108)
276	"	F.	35	Neurasthenia		"	U.	Many	Ferments saccharose	"	Nil
279	"	F.	15	Goitre		"	U.	Few	?	Died	?
280	2. ix. 16	M.	39	Phthisis		"	U.	Few	?	"	?
281	"	M.	36	Sciatica		"	U.	Almost pure	G. = M.	Like meningococcus.	Nil
282	"	M.	27	Epilepsy		"	U.	Large proportion	G. > M.	"	800 (M. i)
283	"	M.	72	Apoplexy		"	U.	Almost pure	G. = M.	Like meningococcus.	100 (M. i, NP 235)
289	"	F.	25	Neurasthenia		"	U.	Large proportion	G. = M.	Tenacious growth would not emulsify	?
290	"	M.	48	"		"	U.	One colony	G. = M.	Like meningococcus.	200 (M. xiv, M. xv)
292	"	F.	46	Retroflexed uterus		Norfolk	R.	"	?	Died	?
295	"	M.	14	Normal		Norwich	U.	"	G. = M.	Like meningococcus.	200 (M. xv)
296	"	F.	14	"		"	U.	Almost pure	G. = M.	Colonies yellow.	800 (M. xiii)
297	"	M.	50	Carcinoma of tongue		"	U.	"	?	Strain lost	?
299	"	M.	9	Normal		Norfolk	R.	Large proportion	?	Adherent	?
305	12. x. 16	M.	31	"		Cambridge	U.	Three colonies	M. = G.	Like meningococcus.	800 (M. ii, M. xiii)
307	"	F.	22	"		"	U.	Large proportion	G. > M.	"	800 (M. i, etc.)
310	"	F.	23	"		"	U.	Almost pure	G. = M.	"	800 (M. xiii)
312	"	F.	22	"		"	U.	Large proportion	G. > M.	"	800 (M. xiii)
315	"	M.	18	"		"	U	Many	Ferments saccharose	"	100 (M. i, M. xiii, NP 108)
317	"	M.	24	"		"	U.	Almost pure	G. = M.	"	800 (M. xiii, NP 108, NP 235)
318	"	F.	16	"		"	U.	Large proportion	G. = M.	"	200 (M. i, etc.)

TABLE I—continued.  
 Details respecting persons yielding colonies in the primary plate which resembled the meningococcus.

No.	Date	Sex	Age	Physical condition	Locality	Urban		No. of colonies on original plate	Sugar reaction	Sub-cultures	Highest agglutination
						Rural	U.				
322	16. x. 16	M.	23	Normal	Cambridge	.	.	Large	G. = M.	Like meningococcus.	800 (M. i, NP 108)
333	"	M.	25	"	"	.	.	Many	G. = M.	"	800 (M. xiii)
334	"	M.	18	"	"	.	.	Few	G. = M.	"	400 (M. xiii)
335	"	M.	16	"	"	.	.	"	G. > M.	"	800 (M. i, etc.)
338	"	M.	18	"	"	.	.	Many	G. = M.	"	100 (M. i, etc.)
339	"	M.	16	"	"	.	.	Almost pure	G. = M.	"	400 (M. i, M. xiii)
341	"	M.	19	"	"	.	.	"	G. = M.	"	400 (M. xiii)
342	"	M.	29	"	"	.	.	Few	G. = M.	"	100 (NP 108)
345	18. x. 16	M.	60	"	"	.	.	Many	G. = M.	"	800 (M. i, etc.)
347	"	M.	33	"	"	.	.	"	G. > M.	"	800 (M. i, etc.)
348	"	M.	28	"	"	.	.	"	G. > M.	"	800 (M. i, etc.)
349	"	M.	27	"	"	.	.	Almost pure	G. = M.	"	800 (M. xiii)
350	"	M.	17	"	"	.	.	"	M. > G.	"	800 (M. i, M. xiii)
351	"	M.	18	"	"	.	.	Many	G. = M.	"	200 (M. i)
354	"	M.	17	"	"	.	.	"	M. > G.	"	800 (M. i, M. xiii)
356	"	M.	18	"	"	.	.	Large	G. = M.	"	800 (M. xiii)
357	"	M.	15	"	"	.	.	proportion	G. = M.	"	800 (M. i)
358	"	M.	20	"	"	.	.	Many	G. > M.	"	800 (M. xiii, NP 108)
359	"	M.	23	"	"	.	.	Almost pure	G. = M.	"	200 (M. i, NP 235)
361	"	M.	28	"	"	.	.	Large	M. > G.	"	800 (M. i, etc.)
362	"	M.	21	"	"	.	.	"	G. > M.	"	800 (M. i, etc.)
366	23. x. 16	M.	30	"	"	.	.	Few	G. = M.	"	?
369	"	M.	14	"	"	.	.	"	G. = M.	"	?
371	"	M.	19	"	"	.	.	Almost pure	G. = M.	"	400 (M. i)
372	"	M.	21	"	"	.	.	One colony	G. > M.	"	800 (M. i)
375	"	M.	15	"	"	.	.	Large	G. = M.	"	200 (M. i, NP 108)
384	"	M.	33	"	"	.	.	proportion	G. = M.	"	100 (M. i, etc.)
386	25. x. 16	M.	23	"	"	.	.	Few	G. = M.	"	800 (M. i, etc.)
387	"	M.	29	"	"	.	.	Many	G. = M.	"	800 (M. i, etc.)
388	"	M.	19	"	"	.	.	Few	G. = M.	"	800 (M. i, etc.)
391	"	M.	29	"	"	.	.	"	G. = M.	"	800 (M. i, etc.)
396	"	M.	26	"	"	.	.	Almost pure	G. > M.	"	800 (M. i)
397	"	M.	20	"	"	.	.	Many	G. = M.	"	800 (M. xiii)
400	"	M.	21	"	"	.	.	Large	G. = M.	"	800 (M. i)
								proportion		Colonies yellow: not tested further	
										Like meningococcus.	

TABLE II.

*Showing results obtained with each batch of swabs taken.*

Date when collected	Where collected	Class of individual examined	Number of strains resembling meningococcus in primary plate	Strains shown to differ by cultivation, etc.	Strains died	Strains examined for agglutinability
<i>1st hundred:</i>						
June 13th— July 10th, 1916	Cambridge, Addenbrooke's Hospital and Cambridge County Tuberculosis Clinic	Eye out-patients and their friends. Tuberculosis patients and their friends	18	6	2	10
<i>2nd hundred:</i>						
July 12th— Aug. 15th, 1916	The same: also 16 swabs at tuberculosis colony	The same	35	5	10	20
<i>3rd hundred:</i>						
Aug. 23rd— Sept. 2nd, 1916	Norwich: Norfolk and Norwich Hospital	Medical and surgical out-patients and their friends	56	16	8	27
<i>4th hundred:</i>						
Oct. 12th— Oct. 25th, 1916	Cambridge: Factory X.	Employees in normal health	41	3	1	37

The proportion of positive results obtained, as shown in Table II, increased very considerably during the course of the investigation; this increase may be partly due to greater proficiency in taking swabs and to greater experience in detecting suspicious colonies. In the third batch, taken at Norwich, over 50 per cent. showed suspicious organisms in the primary plates; this number was considerably reduced on further examination, since many of the strains showed definite evidence of belonging to the *catarrhalis* type of organism rather than the meningococcus.

The analysis of results found in Table III is an attempt to show the influence of age, sex, health and surroundings on the proportion of meningococcus-like organisms found. The highest proportion seems to occur in young adult life, but it is also high in old people.

Amongst children a larger proportion of suspect organisms was obtained, but many of these could be differentiated from the meningococcus by cultivation and sugar tests, so that the number of positives in children was rather lower than in adults.

At every age suspicious colonies appear to be more commonly met with in males than in females.

There is some indication that persons with impaired health carry meningococcus-like organisms more frequently than those in good health, and town dwellers more than country dwellers. But the figures suggesting this cannot be insisted upon, as the batches were taken at different times of the year and in different localities.

The general impression is obtained that a fairly high proportion of people of both sexes and all classes and ages can be shown to carry the organisms in question.

TABLE III.

*Analyses of sex, age, surroundings and physical health of 111 persons, from whose naso-pharynges were obtained organisms resembling meningococci in culture and sugar reactions.*

Age Period	-15		16-35		36-60		61-		All ages	
	Total examined	Positive	Total examined	Positive	Total examined	Positive	Total examined	Positive	Total examined	Positive
Male	43	12 (28 %)	122	49 (40 %)	39	10 (26 %)	14	6 (42 %)	218	77 (35 %)
Female	29	5 (17 %)	71	14 (20 %)	71	13 (18 %)	11	2 (18 %)	182	34 (19 %)
Totals	72	17 (24 %)	193	63 (33 %)	110	23 (21 %)	25	8 (32 %)	400	111 (28 %)

## HEALTH AND SURROUNDINGS.

	Total examined	Positive		Total examined	Positive
Town dwellers	267	85 (32 %)	Normal health	292	76 (26 %)
Country dwellers	133	26 (19 %)	Health impaired	108	35 (32 %)

NOTE. A few of the strains recorded in the above analyses were not fully tested owing to their having died (see Table I).

Certain interesting features may be recorded. The patients at Bourn Tuberculosis Colony were living a completely open-air life, and had been practically isolated from the general population for periods varying from a few weeks to six months. Out of 14 examined, five showed that organisms culturally and serologically resembling the meningococcus could be recovered from their naso-pharynges. From one of the strains occurring in a patient who had been in the sanatorium six months, a serum was prepared which agglutinated well both meningococci of cerebro-spinal origin and other naso-pharyngeal strains.

A number of soldiers, all on service at camps in the country districts around Norwich, attended the hospital for deafness, ear discharge, etc., otherwise they were in good health. Out of 13 examined, eight gave colonies like the meningococcus; of these strains one differed in fermenting saccharose, one was lost without being tested, one resembled *M. catarrhalis* and four gave serological reactions similar to those given by the meningococci isolated from cases of the specific disease.

Amongst the employees at the factory, an interesting point came to light which may be of importance.

The factory contains three workshops fitted with lathes and other machinery. Two of the shops, which may be called A and B, have been built and been in use some years; the ventilation in these two shops is dependent mainly on the opening of windows at the will of the men working there; the cubic space per man, while quite in accordance with regulations, is not excessive. The third workshop, which may be called C, has been built within the last year; the machinery is new, and occupies a much smaller proportion of the cubic space than in workshops A and B; the shop is also much loftier than the earlier ones. Ventilation is effected not only by open windows but also by extracting fans and other modern devices, so that conditions in this respect may be said to be as perfect as possible. The cubic space per man is very large compared with workshops A and B. In the following table a comparison between the results found amongst the employees working in these workshops is shown. There are also added the results found amongst employees in the accounts department, the drawing office and other rooms where work demanding special technical skill is carried out. It may be stated that, on the whole, the employees in these last departments receive higher salaries, and

TABLE IV.

*A comparison between results obtained amongst employees working under different conditions.*

	Ventilation, etc.	Cubic space per man	Number examined	Number found positive
Shop A . . .	Windows: old building . . . . .	682	39	24 (61 %)
Shop B . . .	Windows: old building . . . . .	642	11	6 (55 %)
Shop C . . .	Fans, etc., windows: quite new building	2249	17	4 (23 %)
Accounts Dept., drawing office, etc.	Various: all quite satisfactory: (5 of the employees were women) . . . . .	?	15	1 (6 %)

consequently live in more comfortable and probably more hygienic home circumstances. It should be noted that five of the employees in these departments were women, whereas the employees in shops A, B and C were all men.

It will be noted that a far higher proportion of positive results was obtained amongst employees working in the older, less ventilated workshops, where also cubic space was more restricted.

In view of the smallness of the figures it would be unwise to draw a too definite conclusion, but the results are suggestive.

*Agglutination tests on strains collected.*

Nine monovalent sera were prepared in the manner described above. These sera were utilised for testing the agglutinability of all the cocci obtained during the investigation, and also were tested against certain undoubted meningococci isolated from the cerebro-spinal fluid of patients suffering from cerebro-spinal fever. These latter strains, which were given me by Dr Scott, were chosen somewhat at random but included not only strains serologically like those predominating in the recent epidemics but also strains difficult to identify with either of the two main groups of meningococci.

The sera were prepared from the following strains:

*M I.* A typical example of the serological group of meningococci, which has perhaps been found most commonly in recent epidemics. The titre of the serum prepared was 800. The strain used was obtained from Dr Scott, who has designated it C.S. 16 and has found it typical of Group II.

*M IV.* An example of the same group, shown by absorption tests to be practically identical with *M I*. The titre of this serum was about 600.

*M VI.* An example of the same group. The serum did not give either with the homologous organism or with other members of the same group a sufficiently high titre, *i.e.* it did not give complete agglutination at dilutions above 1-200.

*M XVI.* The serum from this organism was prepared by Dr Griffith and used to test the strains I collected because it was a typical example of another common group of meningococcus. The titre of this serum was about 1000. Dr Griffith calls this strain *M 43*, and regards it as typical of Group I.

*M XIV.* This coccus was apparently closely allied to *M XVI*; the titre was about 800.

*M XV.* This coccus was allied to *M XVI* but showed definite differences when tested by absorption methods.

*M XIII.* This coccus was allied to the group of which *M XVI* is an example, but was not identical with it. The serum gave very variable results with suspensions prepared at different times and of different age.

*NP 108.* Unlike the above, which were all prepared from cerebro-spinal fluid strains, this coccus was obtained from the naso-pharynx of a man of 22 who had been isolated in a tuberculosis open-air colony for six months, suffering from advanced phthisis. The coccus resembled the meningococcus very closely, and gave similar agglutination tests to the strains *M I* and *M IV*. The titre of the serum was about 400.

*NP 235.* This was also a naso-pharyngeal coccus, and was obtained from a woman of 45 years of age in good health, living in Norwich. This coccus also resembled *M I* and *M IV* in its agglutinability. The titre of the serum was about 800.

In Table V are grouped the cross-agglutination tests between the test sera and certain meningococci of cerebro-spinal origin, amongst which are the cocci homologous to the sera.

Clear division into groups is not well established in this series. *M I* and *M IV* appear to belong to one group which gives consistent results with all the sera. *M XV* and *M XVI* also give evidence of being closely related to each other and of a different type from *M I* and *M IV*. But the intermediate strains give irregular results which make any classification impossible. The action upon these meningococcal strains of the sera prepared from naso-pharyngeal strains indicates that the latter were more nearly allied to *M I* and *M IV* than to *M XVI*, but hardly gives a basis for definite grouping.

The testing of a large number of strains by agglutination tests presents many technical difficulties. Chief amongst them is the variability of the coccus. Unless the sera are all prepared ready in anticipation, it is impossible to examine the cocci when recently isolated. On the other hand, if the strains are kept for different periods and then suspensions are prepared and examined all at one time against the necessary sera, it will be found that some of the strains do not agglutinate so well (if at all), as they did when first isolated. If, again, stock suspensions from the cocci are prepared immediately after isolation, it will be found that their agglutinability often increases as time goes on. Unfortunately, the extent to which both these changes may influence results is too irregular to be gauged. In these tests (Tables V and VI) the suspensions were generally prepared soon after isolation, and con-

TABLE V.

*Cross-agglutination tests with 16 strains of meningococcus and monovalent sera prepared with seven cerebro-spinal strains and with two strains of cocci obtained from the naso-pharynx resembling the meningococcus in culture and morphology.*

	M I	M IV	M VI	M XVI	M XIV	M XV	M XIII	NP 108	NP 235
Normal rabbits serum, 1:50	Agglutination at 1:100	Agglutination at 1:100	Agglutination at 1:100	Agglutination at 1:100	Agglutination at 1:100	Agglutination at 1:100	Agglutination at 1:100	Agglutination at 1:100	Agglutination at 1:100
M. i	Highest dilution with complete agglutination 800	Highest dilution with complete agglutination 400	Highest dilution with complete agglutination 100	Highest dilution with complete agglutination 1	Highest dilution with complete agglutination 50	Highest dilution with complete agglutination 1	Highest dilution with complete agglutination 1	Highest dilution with complete agglutination 400	Highest dilution with complete agglutination 800
M. ii	800	400	100	1	100	1	100	400	800
M. iii	400	400	100	1	100	1	1	800	400
M. iv	400	400	100	50	100	1	100	400	800
M. v	800	800	200	1	50	100	400	800	400
M. vi	800	800	200	1	200	400	800	800	400
M. vii	800	800	800	100	200	800	800	800	400
M. viii	800	800	800	400	800	800	800	800	800
M. ix	400	400	100	200	100	100	800	400	200
M. x	200	100	100	100	50	100	200	400	100
M. xi	200	100	100	200	100	200	800	400	200
M. xii	200	100	100	400	100	200	800	200	100
M. xiii	200	100	100	400	50	200	400	400	200
M. xiv	200	100	100	800	800	200	800	200	100
M. xv	200	400	200	800	800	800	800	800	400
M. xvi	50	400	200	800	100	500	200	100	1

c = agglutination complete. ± = agglutination well marked but incomplete. ± = agglutination slight but definite.

sequently there was often considerable delay before agglutination tests with the different sera were carried out. These facts must be borne in mind in reading the results of the action of sera on naso-pharyngeal strains.

Table VI shows the action of the same set of sera on cocci isolated from the naso-pharynx. If this table be compared with the previous one giving the action of the same sera on meningococci of cerebro-spinal origin, it will be found that the results obtained with the naso-pharyngeal cocci can be roughly matched in a large proportion of instances with results obtained with the former. Very many match well with the results obtained with the group of meningococci represented by M I and M IV. There are a few, however, which give little or no agglutination with any of the sera tested.

In considering these results it must be remembered that agglutination with an anti-meningococcus serum does not necessarily imply close relationship with the meningococcus, since other organisms, *e.g.* the gonococcus, may agglutinate well with anti-meningococcus sera. On the other hand, a coccus cannot be definitely established to have no relationship with the meningococcus because it is not agglutinated by any of the sera of a particular series. Other sera might be found, if a sufficiently long search were made, which would agglutinate it well.

For purposes of comparison, in Table VII are given the results of the same set of sera on naso-pharyngeal cocci which could be distinguished by cultural or fermentation tests or both from the meningococcus. Many strains of the *catarrhalis* type are omitted owing to the impossibility of making a suspension suitable for the test.

Very few give any indications of agglutinability. No. 296 is an irregular organism which agglutinates with certain sera but also agglutinates completely with normal rabbit serum in a dilution of 1 : 50.

#### *Absorption tests.*

A positive absorption test with a given coccus is the best proof at present available that this coccus is identical with the meningococcus which produced the serum. The essential feature of the proof is the demonstration that, if a given dilution of an anti-meningococcus serum be brought in contact with a quantity of an unknown coccus which is just sufficient to remove the agglutinins that act on that coccus, it will also no longer agglutinate the homologous meningococcus. The test is carried out differently by different workers. I have followed as closely as possible the method proposed by Gordon and Murray (1915)<sup>1</sup>.

<sup>1</sup> Identification of the Meningococcus, *Journ. Royal Army Med. Corps*, xxv. 411-423.

TABLE VI.

Cross-agglutination tests with 94 strains of cocci, obtained from the naso-pharynx and resembling meningococci, and monovalent sera prepared from seven cerebro-spinal strains and two naso-pharyngeal coccus strains.

Strain	M I		M IV		M VI		M XVI		M XIV		M XV		M XIII		NP 108		NP 235	
	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination
7	c	800	c	800	c	200	c	100	c	200	±	±	c	800	c	800	c	400
12	±	50	±	800	±	1	±	1	±	1	±	±	±	±	±	±	±	±
13	c	800	c	800	c	800	c	800	c	400	c	c	c	c	c	c	c	c
18	c	800	c	c	c	c	c	c	c	c								
20	c	800	c	800	c	800	±	1	c	800	c	c	c	c	c	c	c	c
32	c	200	c	200	c	100	±	50	c	100	c	c	c	c	c	c	c	c
65	c	800	c	800	c	800	±	±	c	200	c	c	c	c	c	c	c	c
70	c	800	c	800	c	800	c	100	c	200	c	c	c	c	c	c	c	c
77	±	1	±	1	±	1	±	1	±	1	±	±	±	±	±	±	±	±
99	c	100	c	800	c	800	c	800	c	200	c	c	c	c	c	c	c	c
101	c	400	c	800	c	200	±	1	c	100	c	c	c	c	c	c	c	c
106	c	100	c	800	c	800	±	1	c	50	±	±	±	±	±	±	±	±
108	c	800	c	800	c	100	±	1	c	100	±	±	±	±	±	±	±	±
109	±	1	±	1	±	1	±	1	±	1	±	±	±	±	±	±	±	±
112	c	100	c	50	c	100	±	50	c	100	c	c	c	c	c	c	c	c
117	c	800	c	800	c	800	±	1	c	800	c	c	c	c	c	c	c	c
122	±	1	±	1	±	1	±	1	±	1	±	±	±	±	±	±	±	±
129	±	1	±	1	±	1	±	1	±	1	±	±	±	±	±	±	±	±
131	±	200	±	200	±	100	c	100	±	50	±	c	c	c	c	c	c	c
136	±	1	±	1	±	1	±	1	±	1	±	±	±	±	±	±	±	±



Organisms resembling *Meningococcus*

TABLE VI—continued.

Cross-agglutination tests with 94 strains of cocci, obtained from the naso-pharynx and resembling meningococci, and monovalent sera prepared from seven cerebro-spinal strains and two naso-pharyngeal coccus strains.

Strain	M I		M IV		M VI		M XIV		M XV		M XIII		NP 108		NP 235	
	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination
281	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
282	-	800	-	800	-	200	-	200	-	-	-	-	-	-	-	-
283	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
290	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
295	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
305	-	200	-	800	-	100	-	100	-	200	-	800	-	400	-	200
307	-	800	-	400	-	200	-	100	-	200	-	800	-	800	-	400
310	-	200	-	200	-	200	-	50	-	200	-	800	-	100	-	100
312	-	200	-	400	-	200	-	100	-	200	-	800	-	100	-	100
317	-	400	-	400	-	200	-	100	-	400	-	800	-	200	-	800
318	-	200	-	100	-	200	-	100	-	100	-	100	-	200	-	100
322	-	800	-	400	-	100	-	100	-	200	-	400	-	800	-	400
333	-	200	-	200	-	100	-	50	-	100	-	800	-	800	-	-
334	-	200	-	-	-	200	-	-	-	-	-	400	-	100	-	-
335	-	800	-	400	-	200	-	100	-	200	-	800	-	800	-	800
338	-	100	-	50	-	100	-	-	-	50	-	100	-	50	-	50
339	-	400	-	200	-	200	-	50	-	100	-	400	-	200	-	200
341	-	200	-	200	-	100	-	100	-	-	-	400	-	200	-	200
342	-	50	-	-	-	50	-	-	-	-	-	50	-	100	-	50
345	-	800	-	400	-	100	-	50	-	200	-	800	-	800	-	800



Organisms resembling *Meningococcus*

TABLE VII.  
*Agglutination reactions of strains which in sub-culture or in fermentation reactions were distinguishable from the meningococcus.*

Strain	M I		M IV		M VI		M XVI		M XIV		M XV		M XIII		NP 108		NP 235	
	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination	Agglutination at 1:100	Highest dilution with complete agglutination
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
60	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
139	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
155	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
164	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
186	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
237	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
244	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
249	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
264	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
276	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
286	c	400	c	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-
315	c	100	c	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Differences from meningococcus  
 Colonies yellow in sub-culture  
 Colonies yellow in sub-culture  
 Colonies yellow; ferments saccharose  
 Colonies yellow; ferments saccharose  
 Ferments saccharose  
 Colonies yellow; ferments saccharose  
 Colonies yellow; ferments saccharose  
 Colonies yellow  
 Colonies yellow  
 Colonies yellow  
 Colonies yellow; ferments saccharose  
 Unduly adherent to media  
 Colonies yellow  
 Ferments saccharose  
 Colonies yellow  
 Ferments saccharose

In this method the serum is diluted to 1 : 25 with saline, and an equal quantity of the standard suspension of cocci, as used in agglutination tests, is added; the mixture is then incubated for 24 hours at blood temperature. While this procedure was found to give satisfactory results with a few of my strains, I did not find that it worked well as a standard test, because in the case of many naso-pharyngeal strains the agglutinins acting on the test coccus were not removed to a sufficient extent, and this removal is an essential feature of the test. Gordon and Murray say: "should the result of an absorption test made in this way be at all doubtful, we then saturate the same serum over again and proceed as before. The first saturation sometimes clears off 'agglutinoids' very nicely from serum." It is evident that they have found the method with one "saturation" satisfactory only sometimes. If, now, as they state, a second "saturation" on the same serum is to be effected (presumably with the same standard suspension) it is difficult to see how they retain the same titration dilutions for the test, inasmuch as the serum must be diluted more than 1 : 100 when the final test is carried out.

To avoid this difficulty, and, while effectively removing the test coccus agglutinins, to employ as small an amount of the coccus in exhausting as possible, I adopted the plan of adding small quantities of a somewhat stronger suspension at definite intervals on three successive occasions, thus utilising the well-known fact that the same amount of suspension will remove agglutinins more effectively if it be added part at a time than if it be added all in one dose.

The details of the method were as follows: small centrifuge tubes were placed in series in a rack. Into each tube 2.5 c.c. of a 1 : 25 dilution of the serum used in the test was placed. Sufficient serum should be left over for use as a control in the unabsorbed state. A convenient number of tests to carry out at one experiment was found to be sixteen. For this number, 50 c.c. of a 1 : 25 dilution of the serum was made up; the 16 tubes required 40 c.c. and 10 c.c. were left over for testing in the unabsorbed state. The suspensions for saturating the serum were prepared in the usual way in the strength of 10 milligrams to the cubic centimetre, and were heated at 65° C. for an hour. Into the first test tube was then introduced 0.5 c.c. of the 10 mg. suspension of the organism homologous to the serum; into the next one or two was introduced 0.5 c.c. of similar emulsions of meningococci serologically similar to the homologous organism; into another tube was introduced 0.5 c.c. of an emulsion of an organism known to be inagglutinable with the serum; then into

the remainder of the tubes was introduced 0.5 c.c. of each of the strains of coccus to be tested. To the serum left over for controlling its effect in the unabsorbed state a similar proportion of phenol-saline was introduced, viz. one-fifth of its bulk. The tubes were then corked and placed in the incubator at 37° C. over night. The following morning the same process was repeated, and again in the evening; on the last occasion, when the final dose of emulsion was added, 1.0 c.c. of phenol-saline was also added to each tube, so that then the serum was fully diluted to 1 : 50. The serum for use in the unabsorbed state was also fully diluted in similar proportion. The titration was made on the following morning. Each of the tubes with suspension was centrifuged, and for each emulsion a rack was prepared containing ten agglutination tubes labelled *a* to *j* (see Table VIII). Into *a* was put 0.5 c.c. of the unabsorbed serum; into *b* was put 0.5 c.c. of the same serum diluted a half (now 1 : 100); into *c* and *d* were put the same amounts of 1 : 200 and 1 : 400 dilutions, respectively. Similar dilutions of the serum, after absorption by the coccus, were placed in tubes *e*, *f*, *g* and *h*. Into *i* and *j* were put the same dilutions as in *a* and *b*. The suspension used for the agglutination tests was prepared by diluting the stronger suspension used for absorbing, by adding four parts of phenol-saline to one of the strong suspensions, thus making a suspension of 2 mg. to the c.c. Into tubes *a*, *b*, *c*, *d*, *i*, and *j*, was put 0.5 c.c. of the coccus to be tested, and into tubes *e*, *f*, *g* and *h*, 0.5 c.c. of the suspension of the meningococcus homologous to the serum. The same process was carried out with the homologous meningococcus, the control cocci, and all the cocci under examination. The racks were then put in the incubator at 55° C. for 24 hours, and afterwards allowed to stand for 24 hours at room temperature before the results were read. In this method the serum is treated with 5 mg. of cocci in 3 c.c. of 1 : 30 dilution for about 15 hours, then with 5 mg. of cocci in 3.5 c.c. of 1 : 35 dilution for about nine hours, and finally with 5 mg. of cocci in 5 c.c. of 1 : 50 dilution for about 15 hours.

This procedure resulted in the satisfactory removal of the agglutinins acting on the test coccus in practically every instance. The amount of suspension used is very small, totalling 15 mg. of culture for 5 c.c. of a 1-50 dilution of serum or 3 mg. per c.c.

In Table VIII are given the results of absorbing M I serum with 30 naso-pharyngeal strains and five controls. These 30 strains all agglutinated with the serum when originally tested (Table I). Nineteen of them were obtained from the last 100 swabs (Nos. 301-400), which were taken from healthy workpeople at Cambridge. These are compared

TABLE VIII. Results obtained by absorbing an anti-meningococcal serum with 30 strains of cocci obtained from the naso-pharynx, compared with those obtained by absorbing the same serum with the homologous organism, other meningococci of a type similar to the homologous organism, and cocci of a different species.

Strain	Serum M I												Result of Absorption
	Without absorption						After absorption						
	v. Test coccus						v. Coccus M I						
	A	B	C	D	E	F	G	H	I	J			
	1:100	1:200	1:400	1:800	1:100	1:200	1:400	1:800	1:100	1:200			
Homologous . . . M I . . .	o	o	o	++	+	+	+	+	+	+	+	+	Complete
Positive controls . . . M IV . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
Positive controls . . . M VI . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
Negative controls													
(Gonococcus)	-	-	+	-	o	o	o	o	o	o	o	o	Negative
(Flavus)	-	-	+	-	o	o	o	o	o	o	o	o	"
NP 108 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
229 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
235 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
245 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
247 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
307 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
345 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
348 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
357 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
362 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
372 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
386 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
387 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
391 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
242 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
335 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
347 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
400 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
204 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
223 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
232 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
259 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
296 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
317 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
322 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
349 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
350 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
354 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
356 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"
358 . . .	o	o	o	++	+	+	+	+	+	+	+	+	"

Naso-pharyngeal strains

Complete or practically complete  
Partial  
Negative

with ten strains from my Norwich cases (Nos. 201-300) and with one (No. 108) from the Bourn Tuberculosis Colony.

Special attention may be called to the strains from healthy work-people. It will be seen from the table that nine of them absorbed the agglutinin completely and four absorbed it partially. From this series of 100 swabs, therefore, 13 per cent. supplied a strain which answered every test for cerebro-spinal meningococci.

Very similar results were obtained by absorbing the serum M IV with the same series of cocci; these results are not shown.

Finally, the effect of saturating the two sera prepared from naso-pharyngeal strains was tested with three cerebro-spinal and several naso-pharyngeal strains. The results are shown in Tables IX and X, which may be considered together.

TABLE IX.

*Results obtained by absorbing a monovalent serum prepared from a naso-pharyngeal strain with three strains of meningococci and fourteen strains of naso-pharyngeal cocci.*

Strain	Serum NP 108									
	Without absorption				After absorption					
	v. Test coccus				v. NP 108 coccus				v. Test coccus	
	A	B	C	D	E	F	G	H	I	J
	1:100	1:200	1:400	1:800	1:100	1:200	1:400	1:800	1:100	1:200
NP 108	c	c	c	±	±	±	-	-	±	±
M I	c	c	c	±	c	±	-	-	-	-
M IV	c	c	c	c	c	c	±	-	±	±
M VI	c	c	±	-	c	±	-	-	±	-
NP 235	c	c	c	±	c	±	-	-	±	-
307	c	c	±	-	c	c	±	-	±	±
322	c	c	±	±	±	±	-	-	±	±
335	c	c	±	-	c	c	±	-	±	-
345	c	c	c	±	c	c	-	-	c	±
347	c	c	c	c	c	c	±	-	c	±
348	c	c	±	±	c	c	±	-	±	±
350	c	±	±	-	c	c	c	-	-	-
357	c	c	c	c	c	c	±	-	±	-
362	c	c	c	±	c	c	±	-	±	±
372	c	c	c	±	c	c	±	-	c	±
386	c	c	c	±	c	c	-	-	±	±
387	c	c	c	±	c	c	-	-	c	±
391	c	c	c	-	c	±	-	-	-	-

TABLE X.

*Results obtained by absorbing a monovalent serum prepared from a naso-pharyngeal strain with three strains of meningococci, a strain of gonococcus and eleven naso-pharyngeal strains.*

Strain	Serum NP 235											
	Without absorption				After absorption							
	v. Test coccus				v. NP 235 coccus				v. Test coccus			
	A	B	C	D	E	F	G	H	I	J		
	1:100	1:200	1:400	1:800	1:100	1:200	1:400	1:800	1:100	1:200		
NP 235	c	c	c	±	±	-	-	-	±	-		
MI	c	c	c	∓	c	c	∓	-	∓	-		
MIV	c	c	c	∓	±	∓	-	-	∓	-		
MVI	c	c	∓	-	c	±	∓	-	-	-		
Gonococcus	c	c	c	∓	c	c	c	∓	±	±		
NP 108	c	c	c	∓	c	c	∓	-	∓	-		
307	c	c	c	∓	c	c	±	-	∓	∓		
322	c	c	c	∓	c	c	±	-	∓	∓		
345	c	c	c	∓	c	c	∓	-	∓	∓		
347	c	c	c	±	±	±	∓	-	∓	∓		
348	c	c	±	∓	c	c	∓	-	-	-		
357	c	c	c	±	c	c	±	-	-	-		
362	c	c	c	∓	c	c	∓	-	-	-		
386	c	c	c	∓	c	c	±	-	±	±		
387	c	c	c	±	c	c	∓	-	∓	-		
391	c	c	c	-	c	c	±	∓	∓	∓		

With these sera it was found to be much more difficult to remove completely the agglutinin not only for the homologous strain but also for the test cocci, and it was found necessary to use for saturating 4.5 c.c. of the strong emulsion against 0.5 c.c. of the serum diluted 1:5; this is equivalent to 9 mg. of cocci per c.c. of a 1:50 dilution of serum. Even with this amount, as is shown in Table IX, the NP strains 345, 347, 372 and 387 left marked amounts of agglutinin for themselves as well as for the strain producing the agglutinin.

In such cases incomplete removal of the latter agglutinin may not indicate that the strain tested is specifically different from the agglutinin-producing strain, but it does indicate that a quantitative inferiority exists in the absorbing capacity of this tested strain as compared with others.

I have not met with the same phenomenon—difficulty in removing completely the agglutinin for the test coccus—among my sera prepared with strains of cerebro-spinal origin, but I am not prepared to erect this

into a general distinctive feature of sera produced with naso-pharyngeal cocci.

It will be seen, however, that the meningococci of pathogenic origin, M I, M IV, and M VI, removed a large portion of the agglutinin for the NP coccus used in producing the serum, as also did those NP cocci which, as shown in Table VIII, removed completely the agglutinin from M I serum.

It will be observed further that, though NP 108 and NP 235 are apparently identical with M I since they absorbed its agglutinin completely, yet they are not identical with each other since the agglutinins they produced varied in their combining capacity for different strains. For example, NP 322 absorbed from serum NP 108 almost all the agglutinin for NP 108 itself, whereas from serum NP 235 it absorbed relatively poorly. On the other hand NP 347 behaved in almost exactly the reverse manner, absorbing NP 235 agglutinin and leaving agglutinin in the case of NP 108. These two strains NP 322 and 347 differed from both NP 108 and NP 235 in failing to absorb the agglutinin for M I (vide again Table VIII).

Results such as these make it difficult to rely upon the absorption test for determining the specific identity of strains of unknown pathogenicity with known pathogenic strains. Positive absorption results may be regarded as unequivocal; but negative results, as in the case of NP 322 and 347, may, as just indicated, be quite compatible with relationship to a pathogenic strain and this relationship may be clearly demonstrable by the use of other sera.

#### SUMMARY.

1. In two areas in the Eastern Counties, Cambridge and Norwich, naso-pharyngeal swabs were taken from 400 individuals, who represented different conditions of health and social circumstances.

The investigation was made at Cambridge between the months of June and October, 1916, and at Norwich during August, 1916.

Owing to the low incidence of cerebro-spinal fever in the two towns during the year 1916 (see p. 249), the general population may be regarded as practically "non-contact" in respect of this disease.

2. As a result of the investigation, strains giving all the cultural and microscopical tests of the meningococcus were obtained as follows:

(a) From 200 swabs taken at Addenbrooke's Hospital during June and July, 1917, mostly from normal people, 30 strains.

(b) From 100 swabs taken at the Norfolk and Norwich Hospital during August, from individuals mostly in impaired health, 27 strains.

(c) From 100 swabs taken in Cambridge during October, from factory employees, who were all in good health and mostly in comfortable circumstances, 37 strains.

3. Such strains were found more often in males of every age group than in females, and in adults more often than in children.

In regard to the influence of health and surroundings, the results of the investigation grouped together show a larger proportion amongst town dwellers than amongst country people and amongst people with impaired health than amongst the healthy.

General conclusions cannot, however, be drawn from these figures because conditions were not always comparable. For instance, the majority of country people were examined in June and July, when positive findings were low.

In examining the employees at a factory, I obtained the strains in a higher proportion from the men working in those shops where air space was more restricted and ventilation less perfectly effected.

4. The 94 strains collected from 400 naso-pharyngeal swabs were tested as regards their agglutinability against certain monovalent sera. These sera were prepared from seven strains of meningococci of cerebro-spinal origin, and from two of the naso-pharyngeal cocci which had been isolated during the course of the investigation. The meningococcal strains used for preparing the sera included strains which had been found identical with others occurring with considerable frequency in cerebro-spinal fluid and capable of being grouped in two main groups; they also included strains which differed from these and were apparently rarer; but there is no reason to assume that every variety occurring in the specific disease was represented.

The results of the tests on the above 94 strains may be classified as follows:

(a) 22 gave no agglutination above 1 : 100 with any of the sera used.

(b) Two agglutinated well with certain sera, but also agglutinated with normal rabbit serum.

(c) 31 agglutinated up to 1-200 or 1-400 with the sera of a certain group (M I-M IV), and in some instances gave similar agglutination with other sera not belonging to this group.

(d) 39 agglutinated with the sera of the group M I-M IV to the full titre of the homologous strains, while with the sera belonging to other groups good agglutination was not often obtained.

This last group of 39 strains (41 per cent. of all tested) appears to be indistinguishable by simple agglutination tests from the M I-M IV group of cerebro-spinal meningococci. The previous group of 31 (33 per cent. of all tested) shows evidence of serological relationship with meningococci of cerebro-spinal origin, but it is not possible on their agglutination tests alone to give an opinion as to their identity with any meningococcal group.

Agglutination tests as a whole indicated that about 74 per cent. of strains collected on account of their resemblance to the meningococcus in culture, gave evidence of relationship to the meningococcus in virtue of their agglutination reactions with anti-meningococcal sera.

5. In order to determine if the absorption test would corroborate the relationship shown by agglutination, absorption tests were done against a serum prepared by inoculation of a cerebro-spinal strain of meningococcus, M I, with all the strains, culturally and by simple agglutination indistinguishable from pathogenic meningococci, which had been collected from the last 100 swabs (normal factory employees, Cambridge). Nine absorbed the agglutinin as well as the homologous coccus and four absorbed it partially.

Therefore, out of 100 normal people who had not been in contact with a case of cerebro-spinal fever, 9 per cent. were shown to be harbouring organisms in their naso-pharynx which were serologically identical with meningococci of pathological origin (cerebro-spinal fluid) and, in addition, 4 per cent., making 13 per cent. in all, were so closely allied as to be doubtfully distinguishable even by the test for absorption of agglutinin.

Similar results were obtained with strains obtained from Norwich.

The above results were confirmed by testing absorption by these cocci from another serum prepared with another spinal strain of meningococcus possessing properties almost identical with M I. I have not investigated absorption with sera prepared with strains which differ serologically from M I.

6. With regard to those naso-pharyngeal strains which were not identified with cerebro-spinal strains by serological tests (agglutination and absorption), I consider that in view of the great variation in the serological reactions shown by different strains of undoubted meningococci and even by different emulsions of the same strain, it is very difficult, if not impossible, to exclude any such microscopically and culturally typical organisms from the meningococcus group on the basis of serological tests.