THE COMPLEMENT FIXATION TEST IN RELATION TO THE GONOCOCCUS AND ALLIED ORGANISMS.

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CONTENTS.

1. Introduction.

The work described in this paper was commenced in 1926 in order to demonstrate whether or no infections with the *M. catarrhalis* afford positive reactions with gonococcal antigens. It had already been shown that an antigen of *M. catarrhalis* might react with the sera from gonococcal infections. Later it was decided to investigate aberrant forms of *M. catarrhalis* and allied organisms and also to test whether sera which react very strongly to the Wassermann reaction might also react non-specifically to the Gonococcal Complement Fixation test, possibly to such a degree as to detract from the value of the latter. Such a non-specific reaction might be of the nature of a group fixation (the Wassermann being not anti-spirochaetal but probably anti-lipoidal), or to adventitious substances introduced in the preparation of the gonococcal extracts from cultures derived from patients with secondary syphilis.

I wish to acknowledge especially my indebtedness to Col. L. W. Harrison, D.S.O., Director of the Venereal Diseases Department of St Thomas's Hospital, for his continual assistance and encouragement during the work of the research and the preparation of this report, and to thank the Curator of the National Collection of Type Cultures, Dr Robert Thomson of the Pickett-Thomson Laboratories and Dr J. Bamforth of St Thomas's Hospital for some of the cultures used in the work.

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2. Cross-fixation experiments with animals.

Strains of *M. catarrhalis* were obtained from several sources. The criteria as to their character being gram negative cocci:

1. Showing a definite degree of autolysis of certain members when cultivated for more than 24 hours.

2. Fermenting none of the ordinary carbohydrates and producing alkalinity in glucose, laevulose, saccharose, dulcite, maltose, lactose and mannite when using phenol red as an indicator.

3. Capable of growth on ordinary nutrient agar both at room temperature and 37° C.

4. Emulsifying fairly readily from agar slopes in saline of physiological strength.

In all 14 strains were used for this portion of the investigation.

Rabbits were used as the experimental animals and here a serious difficulty was met with since it was found that many of their sera were highly anti-complementary, a property which was asserted to the full in the method of complement fixation employed which required prolonged contact of the reagents in the ice-chest. On the other hand a short period of fixation at room temperature or 37° C. was found to produce extremely weak results. Further it was found necessary to raise the amounts of complement to 4 and 5 m.h.d. instead of the 3 and 4 m.h.d. used in testing human sera. This was found to balance the greater absorptive powers of the rabbit sera. It was also found much better to inactivate the animal sera *after* dilution rather than in the neat state.

The sera of animals selected as being suitable on account of low anti-complementary power were first tested against antigens of *M. catarrhalis* and gonococci, using for the former test a pooled extract, produced from ten separately prepared extracts, and for the latter, the routine method of testing for gonococcal complement fixation employed in the department of Venereal Diseases at St Thomas's Hospital, with the addition of the extra complement already described as being necessary whenever animal sera are being tested. Only those animals which gave clearly negative results with both tests were regarded as being suitable for the work.

Technique of testing for gonococcal complement fixation.

The method employed is that used as routine in the Department of Venereal Diseases at St Thomas's Hospital and is based on Thomson's method described in the Medical Research Council special report series No. 19, such modifications as have been made being those adopted by Dr T. E. Osmond, Pathologist to the Department.

Antigen. This is in the form of an extract of gonococci prepared by adding a minimum quantity of $N/5$ NaOH to emulsions of gonococci in order to produce solution, and subsequently neutralising with $N/5$ HCl. An extract

is made from each of a number of strains of gonococci separately, tested for anti-complementary power (which should be low) and finally tested against known negative sera in the presence of 3 m.h.d. of complement. *Half* the amount of antigen found capable of inhibiting haemolysis under these conditions is taken as the unit dose for each tube. Finally, when the titre of a number of separately prepared extracts has been determined in this manner, the extracts are pooled in inverse proportion to their respective titres.

Complement. Fresh guinea-pig serum is used for this purpose and is titrated as in the No. 1 method Medical Research Council's special report series No. 14 for the Wassermann reaction (Harrison's method).

Haemolytic system. This also is similar to that used for the Wassermann test noted above, *i.e.* 3 per cent. sheep's red cells sensitised with 5 m.h.d. of rabbit versus sheep haemolytic amboceptor.

Serum. This is diluted 1/9 with physiological saline after being inactivated for 45 minutes at 55° C. Animal sera are inactivated after dilution.

Setting up the tests. The general arrangement adopted is that described in the Wassermann technique already alluded to. Three tubes are used for each serum to be tested. One acts as a serum control and contains no antigen, its place being taken by additional saline. Of the other two each contains one unit volume (0·1 c.c.) of diluted serum, one unit volume of antigen and one unit volume of complement containing 3 m.h.d. in one tube and 4 m.h.d. in the other tube. The serum control contains 3 m.h.d. of complement also. The tests are allowed to stand in the ice-chest for 18 hours after which one unit volume of sensitised red cells is added and incubation carried out in a water bath at 37° C. These tests can usually be read some 5 minutes after the serum control tubes and an antigen control tube (containing only 2 m.h.d. of complement) have become completely haemolysed.

Technique of Catarrhalis *complement fixation.*

This is strictly comparable with that described for the gonococcus except that in this case the solution of the organisms to form an extract which will act as an antigen is brought about by means of antiformin. Standardisation of the extract is carried out in a similar manner to that described in the case of gonococci and the tests are put up in exactly the same way.

The *catarrhalis* emulsions used for immunising were made from cultures grown on hydrocele agar (*p*H 7·4) for 24 hours at 37° C. Living and dead emulsions were employed, the former being obtained by washing off with physiological salt solution, the latter by suspension in 0·1 per cent. formol-saline. In each case the emulsions were standardised to contain 1000 million organisms per c.c. Live emulsions were of course injected on the day that they were made up, dead ones 24 hours later, and in some cases the dead emulsions were kept in stock form and injected from time to time as required. Some of the strains tested out were injected subcutaneously into one rabbit and intravenously into a second one, whilst in others only the intravenous

method was employed. Injections were carried out at weekly intervals on the following plan:

1st injection	Live emulsions	100 million organisms	
	Dead ,,	250	,,
2nd ,,	Live ,,	300	,,
	Dead ,,	500	,,
3rd ,,	Live ,,	750	,,
	Dead ,,	750	,,
Subsequent injections	Live ,,	1000	,,
	Dead ,,	1000	,,

Before each injection a sample of blood was drawn off and tested for complement fixation with extracts prepared from *M. catarrhalis* and gonococci respectively.

The immunity (judged by complement fixation with homologous extracts) provoked by the 14 strains varied considerably, as is shown in Table I in which the reactions are set out in detail.

Observations on Table I.

From this table it will be seen that immunisation was more slowly brought about by the subcutaneous than the intravenous method of inoculation and after the first few experiments intravenous injections only were used. The subcutaneous injections were carried out in order that a few results might be obtained which would be comparable with those in the human subjects tested out in a somewhat similar manner (see pp. 264 and 265 with Table II).

One of the rabbits inoculated with strain No. 3 died from toxaemia at a period when no immunity had been established. Septicaemia was the cause of death in the rabbit used for strain No. 8 (organisms recovered from the blood stream).

Strain No. 6 did not produce more than a faint complement fixation power in the serum of the animal employed, and it is noteworthy that it failed equally when injected into the patient from whom it had been obtained ("H." in Table II) although this patient was intolerant of large doses of the strain owing to the general reaction produced, a reaction which suggested the injection of a foreign protein.

All the other strains used produced a fully positive reaction (++) to the complement fixation test for *M. catarrhalis* by the 4th injection, except in the case of strain No. 11 which required six injections.

With gonococcal extracts the degree of fixation produced by the *catarrhalis* inoculations was in all cases less than that with the *M. catarrhalis* extracts, only 5 of the 14 strains tested producing strongly positive (++) results to the gonococcal complement fixation test within the limit of the 10 injections; a single plus (+) reaction with gonococcal extract was, however, provoked by 13 of the 14 *catarrhalis* strains within the limits of the experiment

Table I.

Strain No.	Rabbit No.	Complement fixation before		Method of inoculation	Live or dead emulsion	Complement fixation after 1st injection		2nd injection		3rd injection		4th injection		5th injection		6th injection		10th injection	
		M.c.	Gc.			*M.c.*	Gc.	*M.c.*	Gc.	*M.c.*	Gc.	*M.c.*	Gc.	*M.c.*	Gc.	*M.c.*	Gc.	*M.c.*	Gc.
1	B.W. 2	N.	N.	S.	D.	N.	N.	±	N.	+	N.	+	N.	+ +	N.	+ +	N.	+ +	±
	B.W. 1	N.	N.	I.V.	L.	±	N.	±	N.	+	N.	+ +	±	+ +	N.	+ +	±	+ +	+
2	W. 1	N.	N.	S.	D.	N.	N.	N.	N.	±	N.	+	N.	+ +	±	+ +	±	+ +	+
	A.	N.	N.	I.V.	L.	±	N.	±	N.	+	N.	+ +	±	+ +	±	+ +	±	+ +	+
3	G.W. 1	N.	N.	S.	D.	N.	N.	N.	N.	N.	N.	Died							
	Br. 1	N.	N.	I.V.	L.	±	N.	+	No test	+ +	±	+ +	±	+ +	+	+ +	+	+ +	+ ±
4	Br.W. 1	N.	N.	S.	D.	N.	N.	±	N.	±	N.	+	N.	±	N.	+	±	+ +	+
	B.W. 3	N.	N.	I.V.	L.	±	N.	±	±	+	±	+ +	+	+ +	+	+ +	+ +	+ +	+ +
5	W. 2	N.	N.	S.	D.	N.	N.	±	N.	±	±	+	N.	+	±	+	±	+ +	±
	B.W. 4	N.	N.	I.V.	L.	N.	N.	±	N.	+	±	+	N.	+ +	±	+ +	+	+ +	+ +
6	G.W. 2	N.	N.	S.	D.	N.	N.	N.	N.	N.	N.	N.	N.	N.	N.	±	N.	±	N.
	B.	N.	N.	I.V.	L.	N.	N.	No test	N.	±	N.	±	N.	N.	No test	±	N.	+	N.
7	C.	N.	N.	I.V.	L.	±	N.	+	N.	+	N.	+ +	N.	+ +	+	+ +	+ +	+ +	+ +
8	Br.W. 2	N.	N.	I.V.	L.	N.	N.	N.	N.	±	N.	±	N.	Died					
9	Br. 2	N.	N.	I.V.	D./L.	N.	N.	±	N.	+	N.	±	±	+	N.	+	±	+ +	+
10	W. 3	N.	N.	I.V.	D./L.	N.	N.	±	N.	+	±	+ +	±	+ +	+	+ +	+	+ +	+ +
11	D.	N.	N.	I.V.	L.	±	±	±	N.	±	N.	+	±	+	±	+ +	±	+ +	+ ±
12	Br.W. 3	N.	N.	I.V.	L.	±	N.	+	N.	+	±	+ +	±	+ +	No test	+ +	+	+ +	+
13	G.W. 3	N.	N.	I.V.	L.	N.	N.	N.	N.	+	N.	+	N.	No test	±	+ +	±	+ +	+
14	B.W. 5	N.	N.	I.V.	L.	±	N.	+	±	+ +	±	+ +	+	+ +	+ +	+ +	+ +		

Explanation of abbreviations (used throughout report):

N., negative.
S., subcutaneous.
I.V., intravenous.
+ +, complete fixation of 5 m.h.d. of complement.
+, complete fixation of 4 m.h.d. of complement.
±, about 40 % haemolysis with 4 m.h.d. of complement.
±, about 75 % haemolysis with 4 m.h.d. of complement.

(10 injections in 9 weeks). In 7 cases the single plus (+) gonococcal complement fixation reaction appeared after the 6th injection, and strain No. 4 of *M. catarrhalis* produced this strength of reaction with only 4 injections.

From the results recorded it is evident that cross-fixation can be made to occur experimentally but that several large doses of the organisms, preferably in the live state and administered by the intravenous route, are necessary to bring this about.

In order to test the possibility of cross-fixation occurring with a serum of an animal injected with small doses of the organism given over a long period the following experiments were carried out.

Rabbits E., W. 4 and Br. 3, each having a negative reaction to both the gonococcal and the *M. catarrhalis* complement fixation tests, were inoculated

Table II.

Patient	Clinical state or diagnosis	Complement fixation before injection		Details of weekly injections	Complement fixation after injection		Subsequent history of complement fixation: Interval since injection (months)	Complement fixation	
		M.c.	Gc.		*M.c.*	Gc.		*M.c.*	Gc.
P.G.	Chronic nasal catarrh	±	N.	50, 100, 150, 200	+ +	±	3	+ ±	±
				200 on 4 occasions	+ +	+	15	+	±
							24	+	±
H.	Chronic rhinitis	±	N.	25, 50, 100, 100	±	N.	3	±	N.
F. 9694	Syphilis	N.	N.	100, 100, 200	+	±	Not tested		
F. 8528	Syphilis	N.	N.	200, 200	+	N.			
				500	+ ±	±	1	+	±
B. 502	Syphilis	N.	N.	100, 200, 500	±	N.	3	N.	N.
21592	Syphilis	N.	N.	200, 200, 200	+	N.	3	N.	N.
G. 9560	Syphilis	N.	N.	100, 200, 500	+	N.	Not tested		
G. 3022	Syphilis ? old gonorrhoea also	N.	±	100, 200	+	±			
				500	+ +	+			
				500	+ +	+ +	3	+ +	+ +
G. 4688	Syphilis	N.	±	100, 200	+	±			
				500	+ ±	±	2	+	±
M.C.	Normal subject	N.	N.	100, 200, 400	+	N.			
				600, 800	+ +	N.	3	+ ±	±
				1000, 1500	+ +	N.	6	+	N.

subcutaneously with 50 million dead organisms weekly for 3 months and then for a further one month with 100 million of the dead organisms each week. The sera of all three rabbits gave feeble complement fixation reactions with *M. catarrhalis* extract by about the end of the 3 months, the results being ±, + and ± respectively. At the end of the further month the readings to the *M. catarrhalis* fixation test were ±, + and ± respectively. At no time in the course of the experiment did the sera give any complement fixation reaction with a gonococcal extract.

The foregoing experiments were then extended to some patients and a few volunteers. The results of the injections in this series of cases are shown in Table II in which full details of the injections and the results of tests carried out at various times have been noted.

Observations on Table II.

From the results recorded in this table it appears that in the human subject injections of *M. catarrhalis* do not easily provoke such a response as to lead to the serum affording positive reactions with the gonococcal complement fixation test. In only 2 of the 10 patients, viz. No. G. 3022 and P.G., was such a cross-fixation reaction produced of a degree of one plus (+) or stronger.

Of these patients No. G. 3022 gave an apparent cross-fixation so rapidly that the presence of an old gonorrhoeal lesion was suspected. Examination revealed the presence of a considerable amount of pus in the prostate and a heavy infection with secondary organisms. Although no gonococci were found in this patient the result of the examination suggested very strongly an old gonorrhoeal infection which might account for the exceptionally rapid increase of the gonococcal complement fixation reaction, and for this reason it does not seem justifiable to regard the result as conclusive of the power of *M. catarrhalis* to provoke cross-fixation.

Patient P.G. gave a very clear history of complete absence of venereal disease (Wassermann reaction also negative) and the single plus (+) obtained in the gonococcal complement fixation test represents a true cross-fixation. The very long persistence of this reaction is worthy of note.

Thus injections of *M. catarrhalis* by the subcutaneous route into the human subject may lead to some degree of cross-fixation usually small in amount.

3. GONOCOCCAL COMPLEMENT FIXATION TESTS IN *M. CATARRHALIS* INFECTIONS.

Patients who were suffering from definite infections with *M. catarrhalis* or who harboured this organism in one of the secretions were also sought. Great difficulty was experienced in securing any large number since in many of the cases investigated the gram negative cocci found in the nasal or other secretion proved not to be a true *M. catarrhalis*. In all, 22 cases were obtained, and the blood of each was tested for gonococcal and *M. catarrhalis* complement fixations.

The results of the tests in this series are shown in Tables III and III *a*. The case described in Table III *a* is included with the cases harbouring true *M. catarrhalis*, as the organism isolated from this case proved to have very similar characteristics to the typical organisms.

Appendix to Table III a.

Case of patient No. 1151.

History. Patient ran the risk of venereal disease in 1913. Later, in 1926 a small penile lesion occurred which disappeared in a few days. In 1927 the patient presented himself for full tests, complaining at that time of rheumatism of shoulders and knees.

Table III.

	Patient	Diagnosis, clinical state and site of *M. catarrhalis*	Length of history	Presence or absence of history of gonorrhoea, etc.	Complement fixation		Remarks
					M.c.	Gc.	
1	E.P.	Chronic nasal catarrh, *M. cat.* in nasal secretion	Several years	Nil	±	N.	*M. cat.* present in almost pure culture. Few staphylococci in addition
2	S.	Chronic bronchitis, *M. cat.* in sputum	3 years	Nil	+	N.	Enormous numbers of the *M. cat.* present
3	18754	Recurrent colds, *M. cat.* in sputum	7 years	History denied, signs nil, has syphilis	+	+ ±	This case is fully discussed later
4	D.M.	Chronic nasal catarrh with organisms in nasal secretion	5 years	Nil	N.	N.	
5	P.B.	Recurrent colds, *M. cat.* in post-nasal swabs	Many years	Nil	±	N.	
6	E.M.	"Influenza" with organisms in sputum	1 week	Nil	+	±	History of recurrent colds for several years
7	H.	Chronic rhinitis, *M. cat.* in nasal secretion	1 year	Nil	±	N.	
8	C.	Recurrent colds, *M. cat.* in sputum	Years	Nil	±	±	
9	G.	Asthma, organisms in sputum	15 years	Nil	+ +	N.	*M. cat.* present in very large numbers in sputum almost pure
10	E.F.B.	Chronic pharyngitis, organisms in sputum	10 years	All history denied, no signs, no other V.D.	±	±	
11	S.	Recurrent colds, organisms in post-nasal swab	?	Doubtful history of gonorrhoea in past	±	+ ±	This case is fully discussed later
12	G.H.	Recurrent colds, organisms in sputum	?	Nil	±	N.	
13	O.W.	Acute maxillary sinusitis, organisms in pus	3 weeks	Nil	+ +	N.	
14	H.N.E.	Acute coryza, *M. cat.* in nasal secretion	2 days	Nil	±	N.	
15	H.W.	Chronic bronchitis and asthma, *M. cat.* in sputum	5 years	Nil	±	N.	
16	M.B.	Recurrent colds, organisms in post-nasal swab	Years	Nil	+ +	±	
17	J.W.	Cystitis, organisms in urine	1 year	Denied	+ +	N.	
18	A.M.B.	Cystitis, organisms in urine	5 weeks	Denied	±	N.	
19	F.J.S.	Nasal polypi, organisms in nasal secretion	?	Denied and no signs	N.	±	
20	N.	Recurrent colds, organisms in sputum	Years	Acute gonorrhoea 7 years ago	±	±	
21	D.A.	Recurrent colds, organisms in sputum	Years	Denied	+	N.	

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State on examination. All reflexes were active. Gums edged with a white line and soft palate in a condition of leukoplakia with little red dots on the surface.

Examination of blood. Wassermann reaction, Kahn test and Dreyer-Ward Sigma reaction all completely negative.

Gonococcal complement fixation test strongly positive (++).

Subsequent history of the case.

In view of the result obtained in the gonococcal complement fixation test the patient was further questioned in regard to any history of gonorrhoea. This was denied, the patient remarking that if any discharge had at any time been present it was too small in amount to attract attention.

It was, however, found that the patient was suffering from a chronic catarrhal condition of the upper respiratory passages and this had actually caused some deafness.

Table III *a*.

Case No. 22 [1151].

Time of test Details of vaccine etc.	Routine gonococcal complement fixation test	Titre of complement fixation in patient's serum using 3 m.h.d. of complement and standard doses of extract			
		Gc. extract	*M.c.* extract	Aberrant *M.c.* extract	Homologous extract
First observation	+ +	—	—	—	—
Second observation	+ +	—	—	—	—
Third observation: at time of isolating the aberrant *M. cat.* from nasal secretion	+ +	1/80	1/80	1/100	—
After 3 doses of vaccine	+ +	1/60	1/30	1/160	1/60
Further series of 3 doses of vaccine	+ +	1/40	1/25	1/300	1/160
Interval of 1 month, with vaccine	+ ±	1/25	1/100	1/400	1/500
Interval of 1 month, no vaccine	+ ±	1/25	—	—	—
„ „	+	1/20 (bare)	1/80	1/300	1/400

The prostate was massaged and the fluid obtained showed an occasional pus cell and some secondary infecting organisms, but no gonococci either in direct smears or in cultures. The gonococcal complement fixation test was repeated and again found to be strongly positive (++). The nasal secretion was also examined but at this time did not show any *M. catarrhalis*. One week later a second examination of the prostatic fluid was made and this showed some staphylococci but nothing else of pathological import. Urethroscopy showed no abnormality of the anterior urethra. The nasal secretion examined at this time showed an aberrant form of *M. catarrhalis* to be present. It was decided to prepare an autogenous vaccine from this organism and to examine the effect of its administration on the complement fixation power of the patient's serum using extracts of gonococci, *M. catarrhalis*, the homo-

logous extract and an extract composed of a somewhat similar strain of aberrant *M. catarrhalis*.

The method of testing was to find the end-point of fixation of 3 m.h.d. of complement by diluting the patient's serum.

The clinical effect of the administration of the vaccine was some improvement in the catarrhal condition and the results of the series of complement fixation tests are shown in Table III *a*.

Observations on Tables III and III a.

Ignoring the very weak reactions (± and less) which would not be regarded as diagnostic in routine tests, 3 of the 22 cases here reported gave a positive result of diagnostic import in the gonococcal complement fixation test.

In two of these cases the gonococcal complement fixation reaction appeared to be the stronger of the two, whilst in the third case (No. 1151 reported in Table III *a*) the *catarrhalis* complement fixation test appeared to give the stronger reaction.

No. 3 (18754). This patient gave a completely negative history of any previous attack of gonorrhoea and no signs of any such infection could be found on prolonged examination. A venereal history was indicated by the strongly positive Wassermann reaction present in this patient, and the case has been considered in Part 5 of the report from this point of view. Since a very definite *M. catarrhalis* infection was present it would appear to be more reasonable to suppose that the positive gonococcal complement fixation resulted from cross-fixation rather than from a non-specific fixation due to the presence of the strongly positive Wassermann reaction. The gonococcal complement fixation test was found to give a weaker result at a later date.

No. 11 ("*S.*"). This patient gave a somewhat doubtful history of an old attack of gonorrhoea and a few small and fine threads were present in the early morning specimen of urine.

No. 22 (1151, *Table III a*). This case has been fully reported since it is of very considerable interest. It will be seen that the gonococcal complement fixation test was performed in the course of a thorough examination of a patient with a doubtful venereal history. The strongly positive reaction which was obtained could not be considered a true result since no other sign could be elicited on the most careful and complete examination. The positive result was also obtained at a second test carried out to exclude the possibility of experimental error. An aberrant form of *M. catarrhalis* was recovered from the nasal secretion and administered in the form of a vaccine. Such treatment resulted in some improvement in the catarrhal condition which was present and, as is well shown in the table, to an increase in the titre of the patient's serum in complement fixation tests against the homologous extract, a second antigen of like character and to some extent to an extract of true *M. catarrhalis*. The titre with the gonococcal extract fell however at the same

time that the titre to the other organisms was rising, and routine gonococcal complement fixation tests gave a result of $+\pm$ and finally of only $+$ during the course of these experiments (some six months). In view of the very definite history and the complete absence of clinical signs of gonococcal infection this case must be considered as one showing cross-fixation between a serum immune to *M. catarrhalis* (in this case an aberrant form of the organism) and a gonococcal extract.

Thus of the three cases considered in detail two would appear to illustrate the phenomenon of cross-fixation. The third case should be ruled out on account of the doubtful history.

4. CROSS-FIXATION EXPERIMENTS WITH ORGANISMS CLOSELY ALLIED TO *M. CATARRHALIS*.

In view of the fact that *M. catarrhalis* appeared to be much less commonly present in the nose and pharynx than is usually thought to be the case and that, during the search for these organisms, many gram negative cocci were encountered which failed to fulfil the criteria demanded of the typical organisms, it was thought advisable to extend the work to such aberrant forms and to those organisms closely allied to them. The actual identification of some of these organisms presented considerable difficulties since they often failed to correspond with the described characters of certain named and presumably well-defined organisms of the group of gram negative cocci. The recent work of Wilson explains to some extent the reason for this difficulty.

In all, 9 strains were used for animal experiments paralleling those carried out with *M. catarrhalis*. Since the number of strains used was comparatively small and the characters of the organisms differed considerably the one from the other, the necessary extracts were prepared from separate strains and used separately in the different tests. In each case, therefore, the serum of the experimental animal was tested against an extract composed of pooled strains of *M. catarrhalis* of typical characters, against the homologous extract and against another simple extract of an organism presumably identical with the one under test. In addition, the ordinary compound gonococcal extract as used in the routine tests for gonococcal complement fixation was employed.

Considerable difficulty was in some cases experienced in bringing the organism into a suitable suspension for purposes of injection. This was eventually found to be more quickly effected if the organisms were washed from the agar slopes with a minimum of 10 per cent. saline, shaken with sterile beads for 30 minutes and the saline content then reduced to physiological strength by the addition of sterile distilled water.

In order to prevent needless repetition it will be sufficient to indicate the general results obtained rather than to quote the results in full as has been done in the earlier part of the work.

None of the organisms tested by injection into animals gave so marked a cross-fixation as was obtained in the case of the typical *M. catarrhalis*. The

degree of cross-fixation obtainable appeared to be directly proportional to the degree of similarity between the organism under test and typical *M. catarrhalis*. Organisms of the type of *M. flavus* were almost incapable of provoking definite anti-body response even to homologous extracts and therefore no cross-fixation with gonococcal extracts.

As with the work on the typical *M. catarrhalis* so with the present organisms, patients were sought who might harbour the organisms either as carriers without clinical symptoms or as a definite infection. In almost all of these cases however other organisms were present in addition so that the symptoms might equally well be ascribed to other than the gram negative cocci.

Complement fixation tests using extracts of gonoccoci, of *M. catarrhalis* and of the organism actually isolated were carried out.

Of the 14 cases so examined 7 gave no appreciable complement fixation even with the homologous extract. Of the remainder, only one case gave a positive complement fixation with the gonococcal extract and this was a case of cystitis with a history of gonorrhoea. It is of course possible that the original infection was in reality one with the *M. catarrhalis*, in which case no cross-fixation is indicated. The result of the test with gonococcal extract was a weakly positive one and therefore does not provide a good example of the possibility of cross-fixation occurring.

5. Strongly positive Wassermann sera and the gonococcal test.

In carrying out routine gonococcal complement fixation tests occasional positive results were regarded as being non-specific. As these appeared to occur most often in cases with a strongly positive Wassermann reaction it was decided to test out some of these sera and to examine the results in connection with a carefully taken history and a full clinical examination.

Obviously, apart from definite history or signs of gonorrhoea, all patients with a strongly positive Wassermann reaction are suspect, and further, it must be remembered that the time of disappearance of a positive gonococcal complement fixation after cure has never been very definitely determined.

Some of the cases having a strongly positive Wassermann reaction were cases of secondary syphilis, and since such patients may suffer from gonorrhoea at the same time extracts of their organisms may find their way into the antigens used in the gonococcal serum test. It was considered possible (though obviously improbable) that some Wassermann reacting substance (antigen) might be carried over in cultures of gonococci and so lead to the gonococcal antigen reacting with the Wassermann positive serum. Other than this a strictly non-specific factor might be the explanation.

The results obtained in testing 604 sera are shown in Table IV.

Of the four cases two were found to give borderline results between doubtful and weak positive on repeating the test and may therefore be safely neglected.

Table IV.

Total number of Wassermann positive (++) sera tested by the gonococcal complement fixation test		608
Results.	Completely negative reaction	428
	Doubtful reaction (±)	107
	Standard positive reaction (+)	40
	Strongly positive reaction (++)	33
		608

Cases giving a reaction of one plus (+) or more were considered to be reactions which in the ordinary way would be considered as of diagnostic value, and these cases, totalling in all 73, were subjected to further clinical examination and divided into four groups:

Group 1.	Cases which were found either to have a definite gonococcal infection at the time of the test or to give a very definite history of infection in the past	37
Group 2.	Cases giving a suspicious history, or showing some physical signs suggestive of, but not certainly diagnostic of, gonorrhoea	30
Group 3.	Cases which showed no clinical evidence of a gonococcal infection and gave a clear history in no way suspicious	4
Group 4.	Cases which could not be subjected to further examination or cross-examination on account of default	2
	Total ...	73

One case occurred in a congenital syphilitic aged 10 years whose mother had suffered from gonorrhoea.

The fourth case gave a stronger reaction with the *M. catarrhalis* fixation test than with gonococcal fixation test and may therefore have been a case of *catarrhalis* infection. Unfortunately sufficient bacteriological examination to prove or disprove this was impossible.

One must, therefore, admit that no evidence of a falsely positive reaction has been obtained with a serum strongly positive to the Wassermann reaction.

In order to test the possibility mentioned already, that gonococci derived from cases suffering from syphilis in the secondary stage might provide antigens which would react to the gonococcal complement fixation test with sera, strongly positive to the Wassermann reaction, in the absence of a history of gonorrhoea. A number of extracts of gonococci from patients suffering from gonorrhoea in addition to syphilis in the secondary stage were made. A like number of extracts of gonoccoci from cases of gonococcal infection were also prepared; actually six extracts in each group were tested against 50 sera strongly positive to the Wassermann reaction. In addition to a test of the serum with each of the 12 extracts separately, a control test with the ordinary compound antigen used for routine purposes was put up.

The extracts of both groups were found to give comparable results with one another and with the compound antigen, except for individual and slight variation between a clean negative result and some slight degree of inhibition of haemolysis. In no single instance did a gonococcus, derived from a case suffering from syphilis also, afford a diagnostic positive when tested against the sera of patients suffering from syphilis only.

6. Conclusions.

1. That under experimental conditions, in rabbits, a considerable degree of cross-fixation between *M. catarrhalis* anti-sera and gonococcal extracts occurs in complement fixation tests.

2. That the degree of cross-fixation under these experimental conditions is also well marked with aberrant forms of *M. catarrhalis* bearing a close resemblance to the more typical organisms, but is slight with those organisms showing considerable variation from the typical strains.

3. That similar experiments carried out with patients are less successful in producing such cross-fixation, probably owing to the limitation of dosage and method of administration imposed.

4. That naturally occurring infections with the *M. catarrhalis* and aberrant forms of the organism introduce a danger of cross-fixation in gonococcal complement fixation tests. Such results appear, however, to be the exception rather than the rule in such infections.

5. That sera strongly positive to the Wassermann reaction do not tend to react to the gonococcal complement fixation test in the absence of a history or of signs of the disease, and no danger of falsely positive reactions arises as a result of preparing gonococcal extracts from patients suffering from syphilis in the secondary stage in addition to gonorrhoea.

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